ESTIMATES OF THE NUTRITIONAL COST OF THE DEVELOPMENT OF IMMUNITY TO GASTROINTESTINAL PARASITES IN SHEEP

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Abstract

This thesis describes a series of three experiments designed to estimate the nutritional cost of the immune response to the gastrointestinal nematodes *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in sheep. For each experiment, animals were allocated hierarchically by liveweight into one of four groups that were either infected (group IF), similarly infected and concurrently immuno-suppressed with weekly intramuscular injections of 1.3mg kg liveweight $(LW)^{-1}$ of methylprednisolone acetate (group ISIF), immuno-suppressed only (group IS) or remained as controls (group C). Body composition of all animals was estimated using x-ray computer tomography prior to infection and at the conclusion of each study with bodyweight and faecal nematode egg counts (FEC; eggs $gram^{-1}$ of fresh faeces (epg)) measured along with blood samples taken for the determination of levels of serum proteins, phosphate and antibodies.

In the first trial (Chapter 3), the nutritional cost of both the acquisition and maintenance of immunity to gastro-intestinal nematodes was investigated using immunologically naive 5-month-old lambs and immunologically competent 17-month-old ewes during infection with 2,000 and 4,000 L3 infective *T. colubriformis* larvae d^{-1}, respectively (80 L3 *T. colubriformis* larvae $kgLW^{-1}$ d^{-1}). Profiles of FEC and comparative worm burdens at slaughter indicated an effective immune response was maintained in IF ewes and developed in IF lambs while successfully suppressed in both ISIF lambs and ISIF ewes and was confirmed by serum antibody titres. The typical reduction in voluntary feed intake as a consequence of infection was observed in IF lambs

(0.30, $p<0.001$) but not in IF ewes, ISIF lambs or ISIF ewes, and appeared to be associated with L3 IgA. Gross efficiency of use of metabolizable energy (ME) for net energy (NE) deposition was reduced by 0.20 in lambs during acquisition of immunity and by 0.16 in ewes maintaining an established immunity. Infection in immuno-suppressed animals reduced efficiency by 0.05 and 0.15 for lambs and ewes. These findings allowed the hypothesis that the reduction in feed intake and nutrient utilization in young parasitized sheep is caused by physiological signalling associated with the acquisition phase of the host immune response to infection, rather than simply the damage caused by the parasite *per se*.

The second trial (Chapter 4) investigated the influence of metabolizable protein (MP) supply on the metabolic disturbances associated with the acquisition phase of the immune response during infection with 2,000 L3 *T. colubriformis* d^{-1}. Groups of lambs were offered either a low protein (L; 62g MP $kgDM^{-1}$) or high protein diet (H; 95g MP $kgDM^{-1}$). Patterns of total daily egg excretion indicated that an effective immune response was developed in HIF, but not LIF, HISIF nor LISF and was confirmed by comparative worm burdens. The proportionate reduction in feed intake in immunologically normal animals was reduced through the provision of additional protein, being 0.12 in HIF and 0.23 in LIF. Regardless of diet, infection did not cause a reduction in feed intake in immuno-suppressed animals ($p>0.05$). Infection proportionately reduced the gross efficiency of ME utilization in immunologically normal animals by 0.23 in HIF ($p=0.09$) and by 0.51 in LIF ($p=0.01$), but not in immuno-suppressed animals. Immuno-suppression did not suppress serum L3 IgA levels in seven of the eight HISIF and four of the eight LISIF animals. Furthermore, only four out of the eight immunologically normal animals from both the HIF and LIF groups displayed an L3 IgA response. Consequently, regardless of immuno-suppression treatment, animals were termed as IgA responders (HR or LR) or non-responders (HN or LN). Feed intake was proportionately reduced from day 22 by 0.15 in HR ($p=0.03$) and by 0.32 in LR ($p=0.01$), but was not significantly reduced in HN or LN. Gross efficiency of ME utilization was

significantly reduced for LN animals only, being proportionately 0.59 ($p<0.01$). These findings allowed the conclusion that additional MP reduced the consequence of immunological signalling that was displayed in reduced feed intake and in nutrient utilization, both of which appeared to be associated with an IgA response. It is hypothesized that the lessening of nutritional disturbance observed in high protein and immuno-suppressed animals could be a consequence of altered physiological signalling during the immunological cascade.

The third trial (Chapter 5) utilized lambs infected with the abomasal parasite *T. circumcincta* to explore the possibility that the reduction in feed intake and nutrient utilization is a universal phenomenon of the acquisition phase of the immune response to nematode parasites inhabiting different organs along the gastrointestinal tract. In addition, immunological changes at the site of parasite infestation in the abomasal mucosa were measured from serial biopsy tissue samples taken from a further twelve animals that were surgically fitted with an abomasal cannula and either infected (CIF) or concurrently infected and immuno-suppressed as described previously (CISIF). The development of immunity in IF animals was accompanied by a 0.17 proportional decrease in feed intake between days 15 to 28 of infection ($p<0.05$) and a 0.20 proportional reduction in nutrient utilization ($p=0.07$), none of which were observed in ISIF animals. While FEC and worm burdens indicated successful immuno-suppression in ISIF animals, both serum IgA and total antibody production were not reduced. The development of immunity in CIF was reflected in an increase in both mast cells and globule leukocytes in serial abomasal tissue biopsies, both of which were reduced in CISIF ($p<0.01$ for both). In serial biopsy tissue, immuno-suppression did prevent a rise in tissue IgA that was apparent in CIF animals ($p<0.01$) although these changes were not reflected in serum IgA levels. It appears that the alleviation of the reduction in feed intake and nutrient utilization in young lambs through the use of corticosteroid induced immuno-suppression may be a universal phenomenon for both

intestinal and abomasal parasites, but the association with and/or role of IgA during infection with *T. circumcincta* is unclear.

In summary, the reduction in feed intake and nutrient utilization in sheep during infection with both the abomasal nematode *T. circumcincta* and the small intestine nematode *T. colubriformis* appears to be associated with a component(s) of the acquisition phase of the host immune response, rather than, as conventionally assumed, the direct mechanical damage of the parasite *per se*. It is hypothesised that the nutritional disturbance as a consequence of infection in young lambs may be the result of pro-inflammatory cytokines involved in immunological signalling that may also be associated with the production of IgA, the effects of which can be reduced through the provision of adequate MP. These studies provide evidence that the immune response to gastrointestinal parasites is nutritionally costly to the animal and have implications for application of manipulations that are intended to promote the development of a strong immune reaction in high producing animals.

Keywords: *sheep, nematoda, immunity, intake, utilisation of energy, Teladorsagia circumcincta, Trichostrongylus colubriformis, Immunoglobulin A, immuno-suppression*

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Chapter 1
Introduction

Sustainable sheep farming in New Zealand relies heavily on the ability to control and limit the effects of gastrointestinal nematode parasite infections. The ubiquitous presence of nematode larvae on pasture results in the inevitable infection of grazing livestock that represents the major health impediment to high growth rates in intensive farming systems (Niezen *et al*. 1998). Currently, nematode populations are controlled by management strategies that heavily involve the use of anthelmintics to interrupt the parasite life cycle and which appear to be losing effectiveness (Leathwick *et al*., 2001). The ability of the parasite population to quickly develop resistance to a wide range of drugs, in addition to the increasing consumer demand for a more organic product, ultimately means that current strategies for the control of nematode parasites for New Zealand farmers are unsustainable; consequently, alternative methods of nematode control must be found.

Currently, the most likely alternative for parasite control is to increase the animals' ability to mount an effective and natural immune response, thus providing sustainable control while reducing the reliance on chemical intervention. However, the genetic selection of sheep for improved productivity during the last two centuries has resulted in heavier wool weights, faster lamb growth and increased fertility, while providing no apparent increase in fitness in the face of a larval challenge. It may be envisioned that if immunity to parasites was important to facilitate high levels of animal performance, selection for productive traits would result in a corresponding increase in immunological capability. This conclusion is supported by the results of selection trials in which animals that have been selected for productive traits have been found to have a lower immune competence (Howse, *et al*., 1992; McEwan *et al*., 1992; Morris *et al*., 1996), while those that were selected based on their ability to limit nematode eggs in the faeces (immunological ability) have been observed to have lower

productivity (Morris *et al.*, 2000; Kahn *et al.*, 2003; Morris *et al.*, 2005). This indicates considerable diversion of nutrients from productive to immunological processes, and suggests that the immune response may incur a productive cost to the host animal.

This thesis describes a series of experiments that explores the suggestion that the immune response against gastrointestinal nematode parasites in sheep may incur a productive cost, and attempts to separate the metabolic cost imposed by the immune response from that of the parasite *per se*.

Chapter 2

Review of the literature

2.1. Introduction

The use of anthelmintics to interrupt the parasite life cycle (see Bruère and West, 1993; Familton and McAnulty, 1997) and reduce the production losses associated with infection can be considered an unsustainable control strategy. Recently, considerable research attention has been afforded to the development of methods that may enhance the development of immunity in young sheep. This can be achieved either by selection of animals that have increased immuno-competence, or by manipulation of the immune response through vaccination or nutrition, as it has been known for some time that an increased nutritional status of the host helps to guard against the production losses caused by parasitic infection. However, little is known about the effects that increasing the immuno-competence to gastrointestinal parasites will have on the nutrient economy of the host.

2.2. Effects of parasitism on the host

Infection with gastrointestinal parasites can have numerous effects on the host depending on the parasite genera, infection rate and the immunological status of the host animal. Growth rates of lambs during the first 12 weeks of infection with *Ostertagia (Teladorsagia) circumcincta* have been observed to be 13.7, 8.7 and 6.39 kg with infection regimes of 0, 37,500 and 120,000 larvae week^{-1}, respectively, (Symons *et al*., 1981). In comparison, infection with the intestinal parasite *Trichostrongylus colubriformis* for the same period of time resulted in liveweight gains of 14.1, 7.2, 5.0 and -3.9 for animals receiving 0, 3,000, 9,500 and 30,000 larvae week^{-1}, respectively, (Steel *et al*., 1980). Typically, regardless of parasite species, infected animals suffer a reduction in performance through a combination of a reduction in voluntary feed intake and nutrient utilization, both of which are described below, with lamb growth returning to comparable levels to their uninfected controls after the development of an effective immune response and expulsion of the parasite (Kimambo *et al*., 1988).

2.2.1. Feed intake and parasitism

Lower food intake is probably the single most important factor contributing to a reduction in animal performance (Sykes, 1994), attributing to between 40-90% of the losses in production observed during intestinal parasitism (Dargie, 1980; Sykes, 1987). Reductions of 10-30% are typically observed, although it can range in severity from as little as 10-15% to complete anorexia within 1-3 weeks of challenge (Sykes, 1991). The extent of reduction is partly dependant on the numbers of larvae ingested, as although no direct relationship exists, the reduction in feed intake was shown by Steel *et al.* (1980) to be greater in lambs given 30,000 *T. colubriformis* week^{-1} than those receiving 3000 or 9500 week^{-1}, which in turn was greater than those dosed with 300 or 950 larvae week^{-1}. In addition, the effects of mixed infections on feed intake appear to be multiplicative, rather than simply additive effects of single infections with *T. colubriformis* and *O. circumcincta* (Sykes *et al*., 1988).

Despite its importance in the production losses, the reasons for reduced feed intake in parasitised animals are unclear, but appear to be associated with the presence of nematodes in the gut as feed intake has been shown to return to levels comparable to their uninfected controls once animals have developed immunity, or directly after anthelmintic treatment (Kyriazakis *et al*., 1996). Furthermore, reductions have only been observed in ewes during the periparturient relaxation of immunity (Leyva *et al*., 1982). Kyriazakis *et al*. (1998) reviewed several functional hypotheses for reduced feed intake in parasitized animals. He concluded that the two most likely reasons are: 1) anorexia helps animals to promote an effective immune response; 2) anorexia allows the animal to have increased diet selectivity. However, despite these being labelled as functional hypotheses, distinct evidence for either has still not been displayed. Furthermore, in the case of the former, the acceleration of immune development in young lambs that are supplemented with post-ruminal infusions of casein would appear to be contradictory (Bown *et al*., 1991a; Coop *et al*., 1995).

Studies with cattle infected with the abomasal parasite *Ostertagia ostertagi* (Fox *et al*., 1989) have shown a direct relationship between blood gastrin levels and impaired feed intake, with the suggestion that the mode of action may be through altering reticulorumen motility and digesta flow. Additional studies have provided evidence to suggest the involvement of changes in the concentration of the gastrointestinal hormone cholecystokinin (CCK) (Symons and Hennessy, 1981) or in satiety signals from the ventromedial hypothalamus (VMH) (Dynes *et al*., 1990). The role of CCK in control of feed intake is still undetermined, as Samuelsson *et al*. (1996) noted decreases in plasma CCK 36 hours after feed deprivation in cows, concluding that the levels of CCK were stimulated by the presence of food in the gut. No effect on voluntary feed intake in parasitised lambs has been found when CCK antagonist was injected subcutaneously into lambs (Dynes *et al*., 1990; Dynes *et al*., 1998). However, there was an increase in short term feed intake when a CCK antagonist was infused via a cerebral ventricular cannula (Dynes *et al*., 1998). Blocking of satiety signals at the VMH with brotizolam in lambs resulted in a proportional increase in feed intake of 2.6 in parasitized and 1.9 in non-parasitized lambs during the first hour after administration; however, there was no effect on the total feed intake over the next 22 hours (Dynes *et al*., 1990). A further role of the VMH in appetite regulation has been implicated through the activity of pro-inflammatory cytokines produced from an acute phase response (APR). Although specific evidence from sheep is lacking, human and murine studies have identified interleukin (IL)-1, IL-6, IL-8 and TNF-α during bacterial infections that cause inappetence, lethargy and fever-like symptoms (Johnson, 1997; Spurlock, 1997; Johnson, 1998; Langhans, 2000; Farthing and Ballinger, 2001; Colditz, 2002; Colditz, 2004). Together, these findings indicate that VMH receptors may have a role in the satiety signals of parasitised animals and short term manipulation of this may be possible. Further elucidation of the possible role of these is referred to later in the review.

2.2.2. Nutrient utilization

Pair feeding studies have shown the depression of feed intake cannot be totally responsible for the reduced production observed in parasitized animals (Roseby, 1973; Parkins *et al.*, 1973; Sykes and Coop, 1976; Sykes and Coop, 1977), with the remainder in lost productivity attributed to a reduction in nutrient utilization. While dry matter (DM) digestibility has been observed by Coop *et al.* (1982) to be transiently reduced during infection with *O. circumcincta*, a majority of studies have reported either no or very small effects of infection with both *T. circumcincta* or *T. colubriformis* on DM digestion (Parkins *et al.*, 1973; Sykes and Coop, 1976; Sykes and Coop, 1977; Sykes *et al.*, 1988). In contrast, studies with both abomasal and intestinal parasites have consistently been observed to cause a reduction in apparent nitrogen (N) digestibility that has resulted in the daily N balance of infected sheep being 3-5 g less than their uninfected controls (Sykes and Coop, 1976; Sykes and Coop, 1977; Coop *et al.*, 1982). However, it was unclear if the decrease in apparent N digestibility was due to impairment of protein digestion/absorption or as a consequence of an increase in endogenous losses of N. Damage to the acid producing cells of the abomasum by *Ostertagia* species have been observed to cause a rise in abomasal pH either through reduced acid production (Titchen and Anderson, 1977) or through an increase in other secretory products (McLeay *et al.*, 1973; Anderson, *et al.*, 1976; Anderson *et al.*, 1981) that may decrease pepsinogen secretion (Sykes and Coop, 1977) in addition to reducing its conversion to the active proteolytic enzyme pepsin (Matzner *et al.*, 1941). However, reduced N balances are also commonly observed during intestinal parasitism that is not associated with an increase in abomasal pH (Sykes and Coop, 1976; Symons *et al.*, 1981). Pathological damage caused to both the abomasum and small intestine results in increased leakage of plasma proteins into the lumen of the alimentary tract. Despite up to a four-fold increase in albumin turnover rate (Steel *et al.*, 1980), losses in plasma proteins that are not replaced by commensurate synthesis are frequently reflected in a depression of serum albumin concentrations by up to 20g l^{-1} (Steel *et al.*, 1980; Symons *et al.*, 1981;

Coop *et al*., 1982; Poppi *et al*., 1986; Bown *et al*., 1991b). Bown *et al*. (1991b) demonstrated that although 0.87 of leaked plasma proteins were reabsorbed, overall plasma protein loss after seven weeks was increased from 0.68g d^{-1} in controls to 1.97g d^{-1} in animals receiving a concurrent infection of *O. circumcincta* and *T. colubriformis*. These authors also calculated that plasma protein loss into the gastro-intestinal tract only made up 10-36% of the total endogenous losses of N. Furthermore, with the aid of radiolabelled tracers Poppi *et al*. (1986) concluded that the major factor of reduced N balance was the increase in endogenous loss of N, probably from sloughed epithelial cells and mucous proteins.

Even when the majority of the endogenous protein is reabsorbed, there is still poor protein deposition in the body of infected animals, probably because energy is directed to protein synthesis for the preferential repair of the gastrointestinal tissue (Coop and Sykes, 2002). There is in fact, considerable evidence for increased maintenance requirement during infection due to increased gastrointestinal tissue and liver protein synthesis (Sykes, 1991; Sykes, 1997). Yu *et al*. (2000) reported an increase in the sequestration of leucine from arterial pools by the gastrointestinal tract of 24% and an increase in the oxidative loss of 22-41% in sheep infected with *T. colubriformis*. Jones and Symons (1982) observed an increase in the daily fractional synthesis rate of protein in the liver from 0.346g d^{-1} to 0.724g d^{-1} in lambs infected with *T. colubriformis*. In addition, Bermingham *et al*. (2000) showed that infection with *T. colubriformis* resulted in reduced liveweight gain but had no effect on the whole body irreversible loss rate of valine or cysteine, implying repartitioning of amino acids from tissue gain to either repair of intestinal damage or development of immune function. The overall consequence of infection appears to be one of a shift in protein synthesis from protein accretion in both the carcass and wool towards the liver and alimentary tract for the replacement of blood proteins, repair of the gastrointestinal tract and components of the immune response. Such repartitioning will incur an energetic cost in addition to reducing the

availability of scarce amino acids that will ultimately lead to a reduction in the efficiency of utilization of metabolizable energy (ME) for productive functions, which will only be exacerbated when nutrient intake is further reduced by depressions in voluntary feed intake.

2.2.3. Mineral metabolism

Infection with abomasal and small intestine nematodes has been shown to cause a reduction in skeletal deposition of both Ca and P in sheep (Reveron *et al.*, 1974; Sykes and Coop, 1976; Sykes and Coop, 1977; Coop and Field, 1983). In particular, infection with the intestinal parasite *T. colubriformis* can cause extensive villous atrophy that can reduce the absorption of P (Poppi *et al.*, 1986; Bown *et al.*, 1989), resulting in hypophosphataemia and a reduction in P recycling in saliva (Wilson and Field, 1983). The effects of intestinal parasitism on Ca metabolism are still unclear, with increased endogenous losses of Ca (Wilson and Field, 1983) that are reflected in serum hypocalcaemia, but there is no apparent effect of supplementation (Reveron *et al.*, 1974). Bang *et al.* (1990) reported that the elevated abomasal pH as a consequence of infection with abomasal parasites reduced the availability of Cu from Cu oxide wire particles. Generally, there is little information regarding the effect of parasitism on mineral metabolism, as a majority of studies in parasitized young lambs have focused on the disruption to energy and protein metabolism.

2.3. Immunity to Parasites

2.3.1. Mechanisms of the immune response

The immune response of the host against nematode parasites appears to be very complicated, such that many aspects of it are not yet fully understood. Parasites are typically host specific, with complex life cycles and present a variety of antigens unique to each developmental stage. Unlike the immune response against bacterial or viral infection, lambs do not appear to be born with a natural non specific (innate) immunity to gastrointestinal nematode infections (Smith *et al.*, 1985). Immunity to parasites is developed with time

and repeated exposure through an acquired immune response that is slow to develop, seldom provides complete resistance and diminishes with time if challenge is withdrawn (Dineen and Wagland, 1966). Acquired immunity involves the stimulation of both humoral (immunoglobulin (Ig) based) and cellular responses that provide a protection that is specific to both stage of development and parasite genera.

To help explain the observations involved in cellular immunity, the phenomenon has essentially been split into two types of response, either a T-helper (Th)-1 or a Th-2 type response. Upon antigen presentation by a macrophage, antigen presenting cell or dentritic cell, T-helper lymphocytes differentiate into either Th-1 or Th-2 cells, which can be determined by the characteristic profile of the expressed cytokines (communication and/or stimulatory molecules). The type of immune response promoted is ultimately determined by the release of cytokines from the dominant immune response, as the Th-1 and Th-2 type responses are reciprocally regulated (Kopf *et al*., 1993). This model has been successfully used in mouse, rat and human subjects, but the suitability to other species may be questionable. Brown *et al*. (1998) found stimulated T-cells from cattle can express cytokines that are characteristic of both Th-1 and Th-2 responses. In a review of the Th-1 and Th-2 type responses in the regulation of immunoglobulin isotopes in cattle, Estes and Brown (2002) concluded that the classical roles of many cytokines in the laboratory mouse do not extrapolate entirely or at all to cattle. Although there have been very few studies in sheep on the exact cytokine cascade and the role of each component, Gill *et al*. (2000) found evidence for a Th-1/Th-2 dichotomy in sheep infected with *Haemonchus contortus*.

Th-1 responses are generally considered to be an inflammatory and 'innate' action of the immune system, stimulated by the release of IL-12 from the dentritic cells (Jankovic *et al*., 2001). Th-1 cells release the cytokines IL-2, interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α) that

stimulate IgG production and cell mediated responses (Artis and Grencis, 2001) which includes the activation of macrophages to a microbicidal state. Of the many cytokines released by macrophages, the most relevant for the course of this review appear to be the acute phase response (APR) proteins IL-1, IL-6 and TNF-α that act on the fibroblasts and endothelial cells and hypothalamus to cause an inflammatory response and increase in body temperature that causes fever like symptoms (Johnson, 1997; Spurlock, 1997). Th-2 responses are specific, or 'acquired' immune reactions to antigens. Th-2 cells are known to release IL-4, IL-5, IL-9, IL-10 and IL-13 which promote the growth and differentiation of mast cells and eosinophils as well as production of IgE, which is believed to mediate the activation of these cells (Jankovic *et al.*, 2001). Production of IFN-γ from Th-1 cells inhibits IL-4, which is involved in a majority of Th-2 responses (Kopf *et al.*, 1993). Similarly, IL-10 is produced from a Th-2 cell which suppresses the release of IFN-γ from Th-1 cells. Triggering mechanisms for either a Th-1 or Th-2 response appear complex and are not yet fully understood. It has, however been described by Jankovic *et al.* (2001) as balancing on a tightrope between a relatively weak 'innate' response triggering the development of Th-1 cells and sufficient co stimulatory-molecule signalling to promote the Th-2 response.

2.3.2. Cellular and humoral immunity

Antigenic stimulation from parasites in a naïve animal will result in the activation of a pro-inflammatory Th-1 type reaction that evolves to a specific Th-2 type immune reaction as an effective immune response develops, resulting in the formation of a protective immunity. The development of resistance to nematode establishment and expulsion of adult worm burdens are associated with marked cellular infiltration of the intestinal mucosa with mast cells and globule leukocytes (Miller and Jarret, 1971) that are believed to have the same cell lineage (Huntley, 1992), often with large numbers found in immunologically competent animals (O'Sullivan and Donald, 1973; Huntley, 1992; Douch and Morum, 1993; Stankiewicz *et al.*, 1993; Williamson *et al.*, 1994; Winter *et al.*, 1997). Upon being triggered by IgE, mast cells

release vasoactive substances such as histamine and leukotrienes that cause an inflammatory response allowing plasma antibodies into the gut lumen, as well as acting directly on the parasites (Douch *et al*, 1983; McFarlane, 1997). Typically, increases in both mucosal mast cells and globule leukocytes have been associated with immunological protection, however, the number of globule leukocytes has been shown to have a strong correlation (R=0.92) with larval migration inhibition in the mucus following *T. colubriformis* infection in sheep (Stankiewicz *et al*., 1993), presumably acting through the release of the slow-reacting substance of anaphylaxis (SRS-A) (Douch *et al*., 1986).

In addition to the changes in cellular immunity, parasite-induced antigenic stimulation causes a pronounced increase in the production of an array of nematode-specific immunoglobulins. The most abundant of these in ruminants is IgA (Mestecky *et al*., 1999), which has been implicated in the regulation of worm length and fecundity of populations of *O. circumcincta* and *H. contortus* (Smith *et al*., 1983; Stear *et al*., 1999; Strain and Stear, 2001), and levels of which in lymph draining the abomasum can indicate protection against *O. circumcincta* (Smith *et al*., 1987). The role of IgG is unclear, however, phenotypic correlations of up to 0.63 for worm burden and 0.62 for faecal egg count (FEC; eggs gram^{-1} (epg)) with serum IgG levels have been observed (Douch *et al*., 1995). In comparison, IgE is believed to mediate the maturation of mast cells and subsequent production of globule leukocytes (Huntley, 1992). Consequently, serum IgE levels are negatively associated with worm burden, since the expulsion of worms from the gut would result in reduced mast cell turnover that would reduce the requirement for IgE, thus causing a rise in serum levels (Thatcher *et al*., 1989).

In summary, the immune response that is invoked by gastrointestinal nematodes is multifaceted and very complex. As a consequence there are still many gaps in our knowledge regarding the ovine immunological cascade and the role of each component. Furthermore, a majority of our understanding of immune function has been derived from murine studies or

from peripheral measurement of systemic responses in sheep, and it is still not known how reflective these are, both in magnitude and temporally, of what is occurring at the site of nematode infestation.

2.4. Corticosteroids and the immune response

2.4.1. Immuno-suppressive mode of action

Corticosteroids can be classified as mineralcorticoids or glucocorticoids, depending on their pharmacological activity. Mineralcorticoids act primarily on electrolyte and water metabolism, while the action of glucocorticoids includes non-specific anti-inflammatory, immunosuppressant and metabolic effects (Tizard, 2000). This review will focus on the actions of glucocorticoids with reference to their immunosuppressive properties, while bearing in mind their actions on whole body metabolism.

Corticosteroids have been demonstrated to reduce the expression of immunological cytokines from Th-1 (Daynes and Araneo, 1989), Th-2 (Byron *et al*., 1992) or both cell populations (Almawi *et al*., 1991). The action by which depletion of immune function occurs is believed to be common amongst all corticosteroids (Tizard, 2000). Corticosteroids are readily absorbed into lymphocyte cells where they stimulate the production of IκBα protein which suppresses the activities of the transcription factor NF-κB that is responsible for the production of immunological cytokines (Marx, 1995). NF-κB is usually inactivated by being bound to IκBα, however, when a lymphocyte is stimulated the two molecules disassociate and the NF-κB is free to transcribe the RNA sequence of cytokines (Tizard, 2000). When dissociation of this complex occurs upon stimulation during antigenic challenge, the excess IκBα produced as a result of the corticosteroid then binds to the free NF-κB, thus once again forming an inactive complex (Scheinman *et al*., 1995; Auphan *et al*., 1995). This inactivation of NF-κB inhibits the lymphocytes ability to produce cytokines, which in turn ceases the immune cascade.

2.4.2. Suppression of humoral and cellular immunity

The use of the corticosteroid dexamethasone has demonstrated its potent ability to inhibit the immune function of mammalian tissues. Blood eosinophil levels in Romney lambs were shown by Buddle *et al.* (1992) to decrease from 1,000,000 cells ml^{-1} to 50,000 cells ml^{-1} after treatment with dexamethasone that was accompanied by a rise in FEC from 0 epg to 4000 epg. Dexamethasone treatment of sheep for the first 77 days or between days 77-154 of infection by Douch *et al*. (1994) induced FEC in the order of 7000 epg before cessation of dexamethasone treatment, after which FEC reduced to low levels (<100) over approximately 60 days. This pattern of egg production was inversely mimicked by the serum anti-L3 antibody levels, demonstrating that the corticosteroid treatment also inhibited the humoral immune responsiveness of the animals. Further studies by Douch *et al*. (1996a) using a similar trial design demonstrated that abrogation of immunity with dexamethasone also reduces the production of sheep mast cell proteases (SMCP), with lower levels found in sera of animals receiving dexamethasone. Huntley *et al*. (1992b) observed a reduction in SMCP levels in the abomasal mucosa from 3.55mg g^{-1} in animals not treated to 0.4mg g^{-1} in dexamethasone treated animals, that was associated with a reduction in mast cells from 91 to 35 cells 0.2mm^{-2} for untreated and dexamethasone treated animals, respectively, and reflected in respective protection of >90% and <10% from infection with *H. contortus*. In addition, dexamethasone treatment has been shown to increase the mean total worm burden from 17,400 to 340,000 (Jackson *et al*., 1988) and from 473 to 7195 (Adams, 1982), indicating that immune responses to gastro-intestinal parasites in sheep are abrogated with corticosteroid treatment.

In contrast, studies with the use of dexamethasone at doses of 0.5mg kg^{-1}, which are higher than clinical doses, have found a decrease in the worm burdens of *H. contortus* infected sheep when treated around the time of challenge. Adams and Davies (1982) showed a non significant decrease in the abomasal worm burdens from 1,696 to 830 when treated with

dexamethasone at challenge and four days later, and a significant decrease from 3,552 to 1,982 with dexamethasone treatment four days before and at the time of challenge. Similarly, Adams (1983) has reported reductions in worm burdens from 1,937 to 1,123 at day seven, and from 2,073 to 1,277 at day 21 post infection in sheep treated with dexamethasone at four days prior and at challenge with 5,000 *H. contortus* larvae. The reason for this phenomenon is unclear.

2.4.3. Corticosteroid-induced metabolic changes

In addition to their immuno-suppressive qualities, corticosteroids are known to have a net catabolic effect on body protein metabolism. Studies by Paranetto *et al*., (1975) investigated the use of corticosteroids for use as a defleecing agent, with the short-term reduction in wool growth showing a dose-dependant relationship to the point of virtually nil growth when a single dose of 6mg Opticortenol (dexamethasone) kg^{-1} was administered intravenously, or 2.3mg kg^{-1} administered intraperitoneally. Further studies by Paranetto (1979) observed similar effects on wool growth while also reporting short-term reductions in feed intake of up to 100% for eight days following treatment. In contrast, Adams and Sanders (1992) observed up to 30% greater intakes and reduced liveweight loss of 1.5kg during 20 days in sheep that were treated with 0.1mg dexamethasone kg^{-1} during entry into feedlots. Corticosteroid treatment may stimulate intake through increasing the production of a leptin antagonist, neuropeptide-Y, thus reducing the anorectic effects of leptin production on the appetite centres in the hypothalamus (Williams and Mobarhan, 2003, Chilliard *et al*. 2001). Huang *et al*., (1998) reported no effect on feed intake or liver weight but a dose related decrease in weight gain, carcass weight and gastromemius/plantaris muscle mass in rats treated with up to 50mg kg^{-1} of corticosterone acetate. Turini *et al*. (2003) observed a decrease in feed intake on day 2 that recovered by day 4 and was accompanied by a decrease in liveweight in rats after an intraperitoneal injection of 120mg kg^{-1}. These authors also found protein fractional synthesis rates to be decreased in muscle and gut mucosa by 15%

and 19%, respectively, while increased in the liver by 61%, which was reflected in a 17% increase in liver weight of dexamethasone treated rats compared with untreated controls. Bertozzi *et al*. (2000) observed an 11% increase in circulating plasma thyroid hormones in addition to a 50% reduction in plasma concentrations of growth hormone (IGF-1) in calves that received dexamethasone ester implants. Similarly, Thompson *et al*. (1995) found that infusion of cortisol into the bloodstream of sheep significantly reduced the release of IGF-1 when stimulated with growth-hormone-releasing-hormone. Furthermore, dexamethasone has been observed to enhance the deposition of both intramuscular (Dicke *et al*., 1974) and subcutaneous fat (Corah *et al*., 1995; Bertozzi *et al*., 2000).

2.5. Host nutrition and parasitism

The role of host nutrition in reducing the metabolic disturbance of gastrointestinal nematode parasitism presumably through enabling the replacement of endogenous N losses and the development or enhancement of immune responses has been well established and has been extensively reviewed (Sykes, 1987; Sykes, 1991; Holmes, 1993; MacRae, 1993; Sykes, 1994; Coop and Holmes, 1996; Knox and Steel, 1996; van Houtert and Sykes, 1996; Coop and Kyriazakis, 1999; Sykes, 2000; Houdijk *et al*., 2001; Sykes and Coop, 2001; Coop and Sykes, 2002; Kahn, 2003; Sykes and Greer, 2003; Steel, 2003) and may be reflected in the nutrient partitioning framework suggested by Coop and Kyriazakis (1999). A higher plane of nutrition has commonly been observed to result in an increase of resistance/resilience to infection. The distinction between whether nutrition assists either resistance or resilience is unclear, as supplementation trials have demonstrated an increase in resistance shown through enhanced immune development than in their un-supplemented counterparts. However, the development of immunity and subsequent expulsion of the parasite would be expected to result in the animal exhibiting greater resilience in the face of continued larval challenge. For the purpose of this review, the role of host nutrition during infection on resistance/resilience to infection will be considered.

2.5.1. Protein and resistance/resilience

Supplementation studies have demonstrated that supplementation of protein, rather than energy, is required to increase resistance/resilience during infection in both the young lamb (Bown *et al*., 1991a) and in the periparturient ewe (Donaldson *et al*., 1998; Houdijk *et al*., 2000). It is not surprising that dietary protein affects the resistance/resilience during infection, as the young growing lamb has a high amino acid N requirement relative to energy (Ørskov, 1992). However, post-ruminal infusion of casein by Bown *et al*. (1991a) provided evidence that protein supplementation assists the development of acquired immunity, as worm burdens were not reduced by supplementation with 50g metabolizable protein (MP) d^{-1} after six weeks of infection, but were after 12 weeks. This is supported by the studies of Coop *et al*. (1995), who observed greater inhibition of worm development that was associated with an increase in gastric tissue mast cell protease and reduced worm burdens of lambs that received post-ruminal infusions of casein during infection with *O. circumcincta*. Supplementation of three-month-old lambs with up to 100g d^{-1} of fishmeal by van Houtert *et al*. (1995) resulted in increased *in vitro* lymphocyte response to *T. colubriformis* antigen stimulation. Similarly, Kambara *et al*. (1993) also found increased *in vitro* lymphocyte responses to parasite antigens and mitogens from lambs offered a high (20% crude protein (CP)) compared to low (11% CP) protein diets. Knox and Steel (1999) reported greater resilience in lambs when a basal diet of oaten chaff was supplemented with urea that resulted in reduced worm burdens of *T. colubriformis*, but not of *H. contortus*. In addition, abomasal tissue from animals that had received a trickle infection of *T. circumcincta* and were maintained on a diet supplying 180gCP d^{-1} exhibited a greater ability to reject the establishment of L3 larvae *in vitro* than their contemporaries consuming only 83gCP d^{-1} (Jackson *et al*., 2004).

The importance of protein supply to parasitised animals has been shown in diet selection trials, where animals infected with *T. colubriformis* that were offered the choice between high (21% CP) and low (9% CP) protein feeds selected 68% of their diet from a high protein source (Kyriazakis *et al.*, 1994). The effect of selection was that despite a 10% reduction in feed intake, the parasitised animals still achieved a total CP intake similar to that of non-parasitised controls. Further studies by Kyriazakis *et al.* (1996) observed the increased selection by parasitised animals of higher protein diets was eroded by 18 weeks post infection, perhaps indicating a reduced protein demand once their immune response was established.

Condensed tannins (CT) form complexes with plant proteins that decrease their susceptibility to degradation by rumen microbes, thus potentially increasing the protein supply to the animal. In studies where lambs have grazed pastures containing CT (Niezen *et al.*, 1995, Niezen *et al.* 1998; Robertson *et al.*, 1995) or have had CT added to their diets (Athanasiadou *et al.*, 2000; Butter *et al.*, 2000) reduced parasite burdens, lower FEC and increased wool growth have been observed. It is assumed that the greater resistance/resilience to infection is a consequence of a greater protein supply, although there has been some suggestion that the CT may be acting through a direct anthelmintic effect (Athanasiadou *et al.*, 2001). Niezen *et al.* (2002) observed that feeding of the CT containing plant Sulla resulted in reduced worm burdens that were reflective of an increased immunogenic response to *O. circumcincta*, but no effect on the worm burden of *T. colubriformis*, the reasons for the disparity between the two parasite species were not apparent.

2.6. Competition for nutrients

2.6.1. Immunological components

Many components of the immune response such as immunoglobulins and lymphokines are largely proteinaceous in nature (Kambara *et al.*, 1993), the mass proliferation of which would be expected to carry a considerable nutritional penalty (MacRae, 1993; Coop and Holmes, 1996; Coop and

Kyriazakis, 1999), and may explain why additional protein assists the resistance/resilience of infection. The major constituent of SRS-A, which is involved in larval migratory inhibition associated with globule leukocyte production, is cysteine rich leukotrienes (Lewis and Austen, 1981). In addition, Zhou *et al.* (2005) have identified three variants of the ovine IgA allele, with all three having a hinge region that is comparatively cysteine rich. The production of either globule leukocytes or IgA in large quantities during an immune response may result in a reduction of the availability of this sulphur amino acid for other productive functions (MacRae, 1993) and, given that wool synthesis has a very high requirement for cysteine (Rogers *et al.*, 1994), may explain why infection has caused reductions in wool growth of up to 11% in sheep that are resistant to *T. colubriformis* (Barger and Southcott, 1975) and 16% in lambs that are developing an immunity (Sykes and Coop, 1976).

2.6.2. Genetic selection of immune animals

The amount of nutrients invested into immune function may vary depending on both environmental influences and genetic predisposition. In the case of the former, Dionissopoulos *et al.* (2001) reported superior growth rates and feed conversion efficiency in pigs maintained in a minimal disease system. With respect to the latter, evidence for nutrient recruitment by the immune response under genetic influence can be seen in selection trials where animals have been bred to be genetically predisposed to mount a strong immune response (low FEC or resistant lines). Despite the modelling of predicted effects from lambs grazing pastures with lower nematode contamination indicating that superior liveweight gains through selection of lambs with low FEC is possible (Bishop and Stear, 1999), these increases in productivity have not often been realised in New Zealand crossbred animals. Low FEC lines of animals have displayed only slight increases in ewe reproductive performance (Morris *et al.*, 2000) with no (Morris *et al.*, 1997) or even negative (Watson *et al.*, 1992; Morris *et al.*, 2000; Bisset *et al.*, 2001; Morris *et al.*, 2005) effects on post-weaning lamb liveweight gains combined with

increased dag scores (Morris *et al*., 1997). Similarly, lines of animals that have been selected for productive traits including fleece weight, lamb growth rates, or through the use of general productivity indexes have been found to have higher faecal egg counts than control animals (Howse *et al*., 1992; McEwan *et al*., 1992; Morris *et al*., 1996). Additionally, in lines of lambs selected for high or low FEC (Morris *et al*., 1997), there was found to be no difference in the yearling fleece weights between the lines when run separately, being 2.38kg and 2.34kg for high and low FEC lambs, respectively. However, when these lines were reared together there was a highly significant reduction in the wool production of 0.34kg from the low FEC in comparison to the high FEC lines. These results indicate that the greater antigenic stimulation caused by cross-infection from the high FEC lines stimulated a stronger immune reaction in low FEC animals that subsequently incurred a greater metabolic cost that was reflected through the diversion of greater amounts of scarce nutrients away from wool growth. It may be speculated that the nutritional disturbance observed in these animals is primarily through the additional protein required for the mass proliferation of mast cells and globule leukocytes, which have been observed in greater numbers in the abomasal and intestinal mucosa of genetically resistant sheep (Staniewicz, *et al*., 1995; Douch *et al*., 1996b).

2.6.3. The immune response and reduction in intake

The evolution of a non-specific pro-inflammatory Th-1 immune reaction to an acquired parasite-specific Th-2 type reaction, that involves the recruitment of proteinacous cells such as mucosal mast cells, globule leukocytes and immunoglobulins, would be expected to incur a substantial protein and energetic cost to the animal. Consequently, it could be hypothesised that an effective Th-2 type immune reaction would be expected to have a considerable protein requirement, which if not able to be met by the host will result in the increased dominance of a Th-1 type immune reaction. Therefore, it may be expected that gastrointestinal nematode infection in animals in which protein is the rate-limiting nutrient, such as young grazing

lambs or lactating ewes, would result in the promotion of a Th-1 type reaction, leaving the animal susceptible to the consequence of the pro-inflammatory APR cytokine cascade on the hypothalamic appetite centres. This is supported by findings in protein-malnourished mice that were found to have suppressed Th-2 immunity indicated by lower IL-4 and consequently higher IFN-γ levels, resulting in higher worm burdens, lower increases in serum IgE, reduced intestinal eosinophillia and depressed mast cell proliferation after 1, 2 and 4 weeks of infection with *Heligmosomoides polygyrus* (Ing *et al.*, 2000). In addition, Nagasinha (1999) observed 12 month-old animals, which were concurrently immuno-suppressed with the corticosteroid methylprednisolone acetate and infected with *T. colubriformis* while investigating phosphorous metabolism, exhibited no apparent reduction in feed intake or liveweight gain despite FEC of 7,000 epg and worm burdens at slaughter of 267,000, indicating the parasite *per se* may not be responsible for the anorexia and loss of performance during parasite infestation. Furthermore, this model may provide a functional explanation for the inappetence and reduction in nutrient utilization during nematode infections in young animals that is more severe in animals offered low protein diets (Kyriazakis *et al.*, 1996) and which subsides as the development of an effective immune response is established (Kimambo *et al.*, 1988). It is evident that further investigation into the role of immunologically mediated parameters on feed intake and nutrient utilization are warranted.

2.7. Cost of immunity

While a strong immune response against most pathogens or infective agents may be desirable, the wisdom of promoting a powerful immune response in high producing animals has been questioned (Colditz, 2002; Colditz, 2004). Sykes (1994) estimated that the maintenance of immunity to nematode parasites in sheep would incur a 15% loss of productivity, whereas Houdijk *et al.* (2001) estimated the expression of immunity in periparturient sheep to be about 1g kg^{-1} metabolic bodyweight, or 5% of the metabolizable protein requirement. There is still no clear estimation of the nutritional penalty

associated with the development of immunity, although Bown *et al*. (1991a) demonstrated that supplementation with 50gMP d^{-1} was sufficient. Despite this, the consistently poorer performance of animals selected for high immune competence provides clear evidence that a strong immune response against nematode parasites may be counterproductive, which has resulted in attempts to provide a breeding index for resistant/resilient animals (Morris *et al*., 2004). Furthermore, although there were no controls, the extremely large worm burdens of animals that were immuno-suppressed by Nagasinha (1999) while appearing to suffer no ill effects of infection, suggest that the presence of the parasite *per se* caused very little metabolic disruption. Moreover, some cytokines involved in the immune response, particularly those associated with an APR, have been implicated in the depression in appetite, which may add to the cost of immunity.

2.8. Conclusion

Gastrointestinal parasitism in sheep causes a considerable metabolic disturbance primarily through a decrease in feed intake and an increase in protein demand for the replacement of endogenous losses and the development of immunity. However, there is increasing evidence that the metabolic cost of the immune response against intestinal parasites imposes a substantial nutritional burden on the host. It is apparent that an estimate of the cost of immunity is required to help determine the trade-off involved when immuno-competence is included in genetic selection indices, or manipulations that invoke a strong immune reaction, such as vaccination, are imposed on high producing animals. However, given the pathophysiological effects that infection with parasites causes, separation of the effects of the parasite *per se* and that of the immune response will be required. One such method may be through the use of immuno-suppressive corticosteroids.

Chapter 3

The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal parasite *Trichostrongylus colubriformis* on feed intake and utilization in both immunologically naïve and competent sheep

3.1. Introduction

The enhancement of natural immune responses of sheep to reduce the metabolic and productive cost of gastrointestinal parasitism has recently received considerable research attention. Theoretically, given the ubiquitous nature of nematode parasites, host immunity might be anticipated to facilitate productivity and productive genotypes to have stronger immunity than their unselected counterparts. However, attempts to increase the natural immunity by selection of animals maintaining low faecal egg counts (FEC) in the face of larval challenge have not always been associated with the anticipated increase in productivity (Morris *et al*. 2001; Kahn *et al*. 2003). Additionally, sheep selected for production traits such as growth rate and fleece weight have often been observed to have higher faecal egg counts than randomly bred animals (Howse *et al*. 1992; McEwan *et al*. 1992). Together, these findings support a hypothesis that immunity has a significant nutritional cost and that the consequent prioritising of nutrients may compromise productivity (Coop and Kyriazakis, 1999; Colditz, 2002).

Clinical and subclinical intestinal parasitism has been associated with increased endogenous losses of protein into the alimentary tract and increased cost of repair of damage caused by the parasite (MacRae, 1993; van Houtert and Sykes, 1996). In addition, the inevitable reduction in voluntary feed intake limits productivity (van Houtert and Sykes, 1996). Despite suggestions that the reduction of intake may carry some functional role in limiting the effects of parasitism on the host animal (Kyriazakis *et al*. 1998), neither its function nor mechanisms are understood. In a recent study in

which, for other purposes, the immune responses of mature sheep were suppressed with corticosteroids (Nagasinha, 1999), no reduction in intake was observed during infection with *Trichostrongylus colubriformis,* despite the establishment of extremely large worm burdens (>260,000). Furthermore, investigations in murine and human subjects have identified the possibility that cytokines involved in cell signalling during the immune response may disrupt feeding behaviour and growth (Johnson, 1997; Spurlock, 1997). It may be hypothesised, therefore, that components of the immune response, rather than simply the damage caused by the parasite *per se*, may be implicated in the reduction in performance of infected sheep. This chapter describes the effects of immuno-suppression in immunologically naïve and competent sheep during nematode larval challenge on intake, performance and food utilization.

3.2. Materials and Methods

3.2.1. Animals and treatments

Thirty six Coopworth ewe lambs were suckled by their dams at pasture before being weaned and housed at three weeks of age, to minimise nematode larval experience, until the start of the trial at 5 months of age. In addition, thirty six 17-month-old Coopworth ewe hoggets (ewes), which had been subjected to normal farm practices, and were assumed to have developed immunity through natural exposure to nematode larvae, were brought indoors off pasture two weeks prior to the initiation of infection. All animals were drenched with 1ml 5 kg^{-1} liveweight (LW) of a combination drench (37.5g l^{-1} levamisole and 23.8g l^{-1} albendazole, Arrest, Ancare New Zealand Ltd, Auckland, New Zealand) when removed from pasture. Animals within each age cohort were allocated hierarchically by fasted LW into one of four groups (n=9), mean LW 26.6 ± 0.62 and 47.6 ± 1.47 kg for lambs and ewes, respectively. One group of each cohort was infected with the equivalent of 80 L3 *T. colubriformis* larvae $kgLW^{-1}$ d^{-1} (IF), a second group (ISIF) received the same infection but with immune function suppressed by weekly intramuscular injection of the glucocorticoid methylprednisolone acetate, (Depredone, 40mg methylprednisolone acetate ml^{-1} Jurox Pty. Ltd., Rutherford, NSW, Australia) at a rate of 1ml $30kgLW^{-1}$. This regime had been shown previously by Nagasinha (1999) to be successful in preventing establishment of immunity in sheep without compromising animal health. A third group received only the glucocorticoid (IS) and the fourth remained as a control (C), creating two 2x2 factorial designs. The experimental design is given in Table 3.1.

Table 3.1: Experimental design: 5-month-old lambs and 17-month-old ewes were infected (+), or not (-) for 78 days with the equivalent of 2000 or 3200 L3 *T. colubriformis* larvae d^{-1}, respectively, with normal (-) or suppressed immunity (+)

Group	No.		Immuno-suppressed	Infection
	Lambs	Ewes		
C	9	9	-	-
IF	9	9	-	+
ISIF	9	9	+	+
IS	9	9	+	-

3.2.2. Feeding and sampling

Animals were offered fresh water and a complete pelleted ruminant ration *ad libitum* daily (Table 3.2) that was formulated to supply 50g metabolizable protein (MP) d^{-1} in excess of the animals theoretical requirement for 100g d^{-1} LW gain (AFRC, 1993) while consuming daily dry matter (DM) intakes of 3% LW. Individual feed refusals were collected and weighed weekly. Sub-samples of feed offered and refused were taken for determination of DM after drying for 72h at 90°C. Liveweight was recorded at weekly intervals. Fasted LW (after 24h of food deprivation) was also measured on days –7 and 77 of infection to aid the estimation of body composition by computed tomography (described later). Blood samples were taken every two weeks from day -7 using jugular venipuncture into a 10ml vacutube (Becton Dickinson, VACUTAINER Systems, Rutherford, New Jersey, U.S.A) and immediately placed at 4°C for 24h. After centrifugation at 2500rpm for 20min serum was separated and stored at –20°C. Rectal temperature was measured every two weeks.

Table 3.2: Diet composition (g $kgDM^{-1}$) and analysis for the complete rations offered *ad libitum* to 5-month-old lambs and 17-month-old ewes

	Lambs	Ewes
Fish meal	100	-
Molasses	50	50
Barley	426	100
Lucerne Chaff	400	292
Broll	-	540
Salt	1.75	2
Dicalcium phosphate	11	5
Potassium carbonate	8.5	8.5
Magnesium oxide	0.75	0.5
Mineral Mix†	2	2
Analysis		
CP (g $kgDM^{-1}$)	205	174
MJME $kgDM^{-1}$	10.1	8.5
Crude Fibre	146	158
MP supply (g $kgDM^{-1}$)	101	68
DTUP5‡	33.1	7.4
RP5§	130	127

CP = crude protein, MJME = mega joules of metabolizable energy

MP = metabolizable protein

† 1kg contains: 5MIU vit A, 1MIU vit D, 7500IU vit E, 1g Co, 1g I, 20g Mn, 26g Fe, 25g Zn, 210g Mg, 4g P, 0·25g Se, 560g Ca

‡ Estimated digestible true undegraded protein at 5% rumen outflow rate (Agriculture and Food Research Council (AFRC), 1993)

§ Estimated rumen degradable protein at 5% rumen outflow rate (AFRC, 1993)

3.2.3. Parasitology and slaughter/necropsy procedure

Lambs (groups IF and ISIF) were infected with the equivalent of 2000, and ewes with 3200 L3 *T. colubriformis* larvae d^{-1} (80 L3 larvae kg initial LW^{-1} d^{-1}) in three doses each week from day 0 until day 72. The larvae were pipetted from an aqueous solution of known larval content onto filter paper, which was then rolled and administered using a balling gun. Faecal samples were taken directly from the rectum of each sheep at weekly intervals from day –6. FEC were measured using a modification of the McMaster method (Ministry

of Agriculture, Fisheries and Food, 1979) and expressed as eggs g^{-1} of fresh faeces (epg). Infected lambs and ewes were fasted for 24h before slaughter on days 77 and 78, respectively. Slaughter and worm recovery in the small intestine was achieved using methods described by Donaldson *et al.* (2001). Briefly, slaughter was achieved by stunning with a captive bolt followed by exsanguination through severance of the carotid arteries and jugular veins. The abdomen was immediately opened along the midline, the gastro-intestinal tract removed, and the first 6m of small intestine distal to the pylorus ligated and removed, thus holding the intestinal contents within. Small intestinal worm burdens were determined based on the method of Robertson and Elliot (1966). The intestine was gently flushed with tap water and the washings collected and made up to 2l, from which a 10% sub sample was collected and stored in a 10% formalin solution. Worms were recovered from the tissue after digestion in HCL/pepsin solution using the method described by Herlich (1956). Total collection of worms residing in the digested tissue was achieved by washing through a mesh sieve with a 45µm pore size that was sufficient to allow digested material to pass though, while retaining worms. Counting was achieved using 10% aliquots measured in duplicate using a stereomicroscope.

The length of 20 male and 20 female worms was determined using a similar technique to that described by Donaldson *et al.* (2001). Worms chosen at random were placed into a drop of tap water on a microscope slide, onto which a cover slip was placed. The microscope slide was placed onto the stage of a stereomicroscope with a video camera attached to the camera port. The worm image was projected onto a screen through a data projector, the length of which was measured using a map wheel. Calibration was achieved through the measurement of the projected image of a 1mm stage micrometer, the value of which was used to calculate the length of projected worms (mm).

3.2.4. Serum analysis

T. colubriformis-specific L3 total antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) similar to that described by Douch et al (1994). Antigen coated plates were sourced from R.S. Green, AgResearch, Walaceville, New Zealand. Briefly, each well of Costar microplates (Corning Incorporated, U.S.A) was coated with 100µl secretory/excretory antigen (AgResearch, Wallaceville Animal Research Centre, New Zealand) of *T. colubriformis* (2µg ml^{-1}) and left overnight at 4°C before washing with 0.05% Tween 20 in 10mM phosphate buffer, pH 7.2 (PBST) and then blocked with 200µl bovine skim milk powder for 5 min. Plates were then stored at –20°C until required.

The procedure for L3 total antibody and L3 immunoglobulin (Ig) A testing was carried out as described by Xie *et al*. (2004). For total antibody testing, 100µl of a 1:200 dilution of serum was added in triplicate to the wells and incubated at room temperature for 2h. Plates were then washed in PBST, after which 100µl of a 1:4000 dilution of horseradish peroxide (HRP) conjugated rabbit anti-sheep immunoglobulins (Lot: Ch-B 069(101), DAKO, Denmark) was added, and incubated at room temperature for a further 1h. After a further wash in PBST, 100µl of 400µg ml^{-1} o-phenylenediamine (OPD, Sigma) in citrate buffer pH 5.0 and 0.03% H_2O_2 was added as a substrate. The colour was developed in the dark for 12 min before the reaction was stopped with 100µl of 1.5M H_2SO_4. The plates were read at 492nm using a microplate reader (Floustar, BMG Lab Technologies, Germany). Results were expressed as the mean optical density value of the triplicate wells, and adjusted according to a standard serum sample present on each plate. The same methodology was used for IgA antibody measurement, with the exceptions that serum samples were diluted to 1:10, the conjugated antibody was replaced by a 1:2000 dilution of HRP-conjugated rabbit anti-sheep IgA (Lot: A130-108P-3, Bethyl Laboratories Inc, U.S.A) and the colour was developed for 40min.

Serum urea, phosphate, albumin and total protein were analysed on a Cobas Mira Plus Auto-analyser (Roche Diagnostics GmbH, Mannheim, Germany). Serum urea was measured utilizing the urease/glutamate dehydrogenase enzyme system (Urea 5 reagent kit #1489364 Roche Ltd, Basel) with a kinetic UV assay. Serum phosphate was analysed from the presence of an ammonium phosphomolybdate complex (Phos kit#1489348, Roche Ltd, Basel) that was photometrically determined in UV light (340nm). Serum albumin was determined by colorimetric assay utilizing bromcresol green with concentrations determined photometrically (Albumin plus kit #1970569, Roche Ltd, Basel). Serum total protein was analysed by colorimetric assay utilizing the biuret method (Total protein kit#1553836, Roche Ltd, Basel) that was determined photometrically.

3.2.5. Body composition

Changes in the bone, muscle and fat content of the carcass were estimated *in vivo* using x-ray computer tomography on days –14 and 76 of infection. Animals were fasted for a minimum of 12 hours before sedation with an intramuscular injection of 1.0ml 50kgLW^{-1} containing 13.5mg ml^{-1} Acepromazine maleate, ('Acezine 10' Delvet Pty Ltd, NSW, Australia) not less than 30min prior to scanning. Specimens were then restrained in a cradle as described by Nsoso (1995) in order to minimise movement. Scanning procedure was similar to that of Young *et al*. (1996), whereby anatomical reference x-ray cross sections were taken from each animal at the thoracic vertebrae 8, lumbar vertebrae 5 and ischium. This method has been shown to provide accurate and repeatable estimates for carcass fat and muscle but not bone (Young, *et al*., 1996; Lambe *et al*., 2003). In addition, ten animals (five lambs and five ewes) were randomly selected on each occasion for estimation of total carcass tissue weights using the calvaleri principle of Gundersen and Jensen (1987) that consisted of 16-20 equidistant x-ray cross sections taken at 50mm intervals along the long axis, with the initial section taken at random in the neck. The volume of fat, muscle and bone in each slice was multiplied by the distance between slices to give total carcass

volume of fat, muscle and bone. Tissue weights in the carcass were calculated by multiplying the volumes of each by standard density values of 0.925, 1.031 and 1.549 for fat, muscle and bone, respectively, (Nsoso, 1995). This method has been reported to have a 0.95 accuracy (Gundersen ad Jensen, 1987). Carcass weights (CW) for all animals at both scanning times were estimated using the formula: *CW (kg) = (0.6206 X fasted LW) – 3.6182*, that was derived by regressing the actual CW of those slaughtered with fasted LW on day 76 (R^2=0.97). As anticipated, bone represented in the three reference slices did not correlate well with carcass bone found using the calvaleri procedure (R^2=0.13). Consequently bone weight in the carcass was estimated using the following equation derived from Fourie *et al*. (1970): *Bone wt (kg) = 0.2491 X* $CW^{0.7321}$ and then subtracted from the CW to give bone-free CW. Proportions of fat and muscle in the three reference slices did correlate well with proportions of fat and muscle found in the bone-free CW using the calvaleri procedure (R^2=0.94, for both), and were corrected accordingly using the following equations: *calvaleri fat % = (1.0274 X fat % in the reference slices) – 0.63*; *calvaleri muscle % = (1.0274 X muscle % in the reference slices) – 0.0211* to give the proportions of fat and muscle in the bone free CW. The proportions of fat and muscle were then multiplied by the bone free CW to give total fat and muscle weights in each individual. The energy deposited in the carcass gain was calculated assuming muscle tissue consisted of 0.20 protein using energy values of 38.9 and 22.2MJ kg^{-1} for fat and protein, respectively (Blaxter and Rook, 1953).

3.2.6. Wool production

Animals were shorn on days –7 and 77 of infection, and greasy fleece weight recorded at the latter used as a measure of total wool production. A sub-sample of mid-side fleece wool was taken at shearing from each animal on day 77 and stored at 20^{o}C with 0.65 relative humidity until commercially scoured for estimation of clean fleece weight. Energy deposition in the fleece was calculated assuming 23.7MJ kg^{-1} clean fleece weight (AFRC, 1993).

3.2.7. Feed digestibility

An additional eight male hogget rams were housed in metabolism crates to determine digestibility of the diet offered to the lambs. Once adjusted to the diet, four animals were treated with methylprednisolone acetate as described previously. All animals were offered *ad libitum* access to the diet during the following eight days. Feed refusals and faeces production from each animal were weighed and bulked daily, before storage at 4°C. Sub samples of feed offered, refused and faeces were dried to a constant weight at 90°C in a forced air oven. The animals were then adjusted to the diet offered to the ewes and the procedure repeated.

3.2.8. Statistical analysis

Data from lamb and ewe groups were analysed as two separate 2x2 factorial experiments using GENSTAT statistical package (Lawes Agricultural Trust, 2001). Faecal egg counts and worm burdens were log transformed (log10 (count + 1)) before analysis. Worm burden, worm lengths, wool production, digestibility and carcass composition were analysed by ANOVA. All remaining measurements underwent sequential comparison of ante-dependence structures for repeated measures before being analysed by REML with estimates of missing values.

This experiment was carried out with approval from, and in accordance with the Lincoln University Animal Ethics Committee: Authority LU39/01.

3.3. Results

Of the nine lambs allocated to group ISIF, one (No 55) was suspected of not responding to the immuno-suppressive treatment. Parasite specific L3 IgA measurements, (Figure 3.8) indicated incomplete immuno-suppression; consequently this individual was excluded from means and statistical analyses, and its data provided separately. One lamb from group IF died on day 38 and one from group IS on day 74. Post mortem examination revealed the cause of death to be pneumonia and pulpy kidney, respectively. Data from these animals were included in statistical analysis using missing value estimations.

Faecal DM was not measured, however it was observed that no ISIF lamb or ewe had softened faeces or displayed any clinical signs of parasitism at any stage.

Mean rectal temperature was 38.7 ^{o}C ± 0.12 for lambs and 38.4 ^{o}C ± 0.09 for ewes. There was no treatment x time interaction in either group ($p>0.05$ for both), nor was there any effect of treatment on rectal temperature.

Dry matter digestibility was not affected by immuno-suppression, being 0.68 ± 0.01 and 0.58 ± 0.04 for the diets offered to lambs and ewes, respectively.

Liver weights of IF lambs at slaughter were lighter than those of ISIF lambs (798 ± 108 and 899 ± 92 g), respectively ($p<0.05$), but though showing the same trend, were not different in ewe groups (823 ± 145 and 915 ± 138 g) for IF and ISIF ewes, respectively ($p>0.05$).

3.3.1. Feed intake

Feed intake of lambs and ewes is shown in Figure 3.1. For lambs, there was a significant treatment x time interaction ($p<0.01$) reflecting a proportional 0.30 reduction in intake of IF lambs from day 21 of infection ($p<0.001$), before recovery by day 63. In contrast, intake of ISIF lambs was similar to that of C and IS groups ($p>0.05$). Sheep No 55 from the ISIF lambs displayed a similar pattern of intake to that of IF lambs. There were no treatment x time interactions for DM intake in ewes, nor were mean DM intakes different, being 2.01, 2.10, 1.96 and 2.09kgDM d^{-1} for ISIF, IS, IF and C ewes, respectively ($p>0.05$). However, temporary relative reductions of 0.09 ($p<0.05$) and 0.14 ($p<0.05$) were observed in IF compared to C ewes on days 21 and 77, respectively.

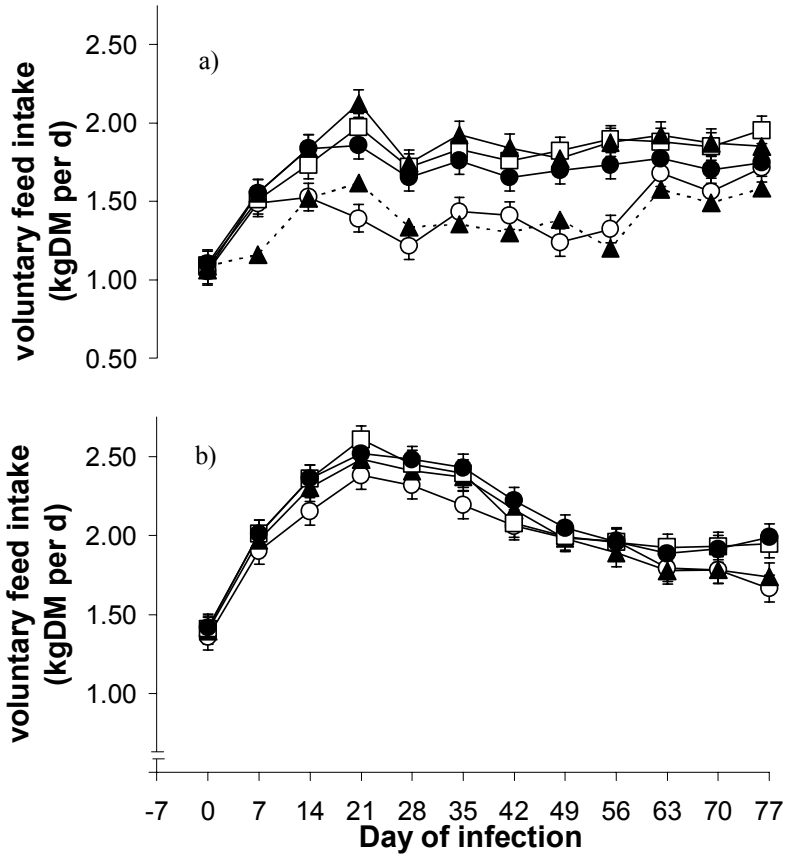

Figure 3.1: Mean daily voluntary feed intakes for a) 5-month-old lambs and b) 17-month-old ewes while ○ infected with 2000 or 3200 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS), □ controls (C), and --▲-- animal No55

3.3.2. Liveweight

Changes in mean LW of lambs and ewes are given in Figure 3.2. There was a significant treatment x time interaction amongst groups of lambs ($p<0.001$). Lambs in group IF were significantly lighter than those in group C ($p<0.05$) at day 35, a difference which increased progressively until IF had 0.16 lighter fasted LW at day 77 ($p<0.001$). Liveweight gain was lower in uninfected immuno-suppressed lambs, with group IS being significantly lighter than group C from day 49 onwards ($p<0.05$), and 0.12 lighter on day 77 ($p<0.01$). Liveweight of ISIF lambs was at all times comparable with, and not significantly different from that of IS ($p>0.05$). Impairment of growth due to infection was prevented by immuno-suppression, with ISIF lambs being 0.09 heavier than IF lambs between days 56 and 70 of infection ($p<0.05$). In ewe groups, there was no treatment x time interaction ($p>0.05$), though ISIF ewes did have a 0.08 lighter fasted LW than C ewes on day 77 ($p<0.05$).

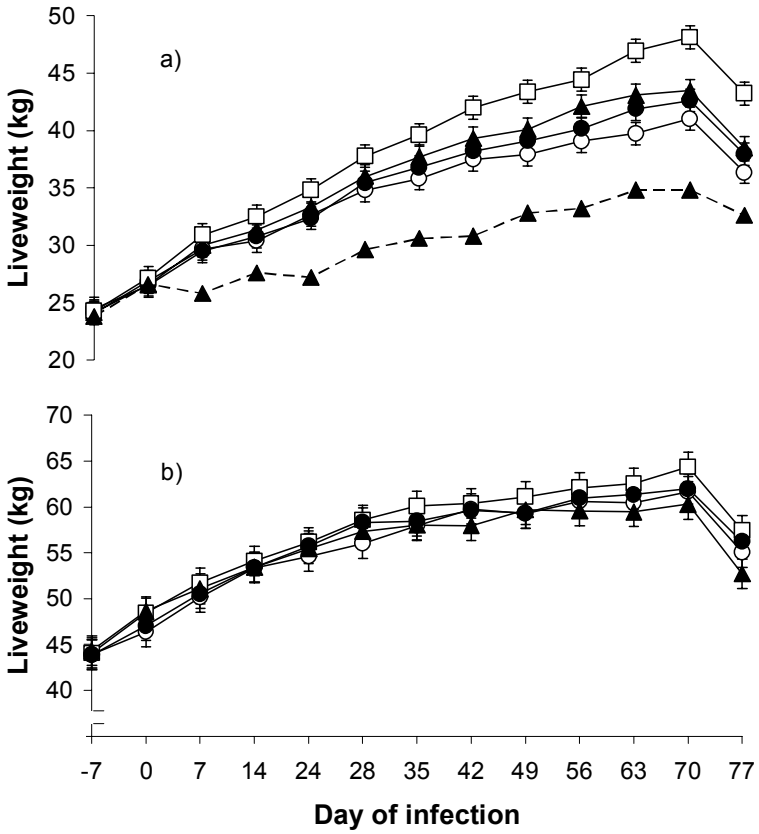

Figure 3.2: Mean liveweights of a) 5-month-old lambs and b) 17-month-old ewes while ○ infected with 2000 or 3200 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS), □ controls (C), and ⁻⁻▲⁻⁻ animal No55. Values for days -7 and 77 are 24h fasted liveweight.

3.3.3. Faecal egg counts

The mean FEC of lambs and ewes are shown in Figure 3.3. Eggs were detected from day 20 of infection in IF lambs, and peaked at 1250 epg on day 41 before declining to less than 100 epg by day 75. Lambs in group ISIF showed a similar trend in FEC to group IF during the first 41 days of infection but thereafter FEC continued to rise and were greater than in IF (plateau of about 4000 epg) on day 62 ($p<0.001$). Eggs were not detected in uninfected groups of lambs. Eggs were not observed in the faeces of IF ewes until day 41 of infection. Counts of less than 100 epg were then maintained until the conclusion of the trial. In contrast, the mean FEC in ISIF ewes was significantly greater than in IF from day 27 onwards ($p<0.01$), with counts approaching 4000 epg by the conclusion of the trial. Eggs were not found in uninfected ewes.

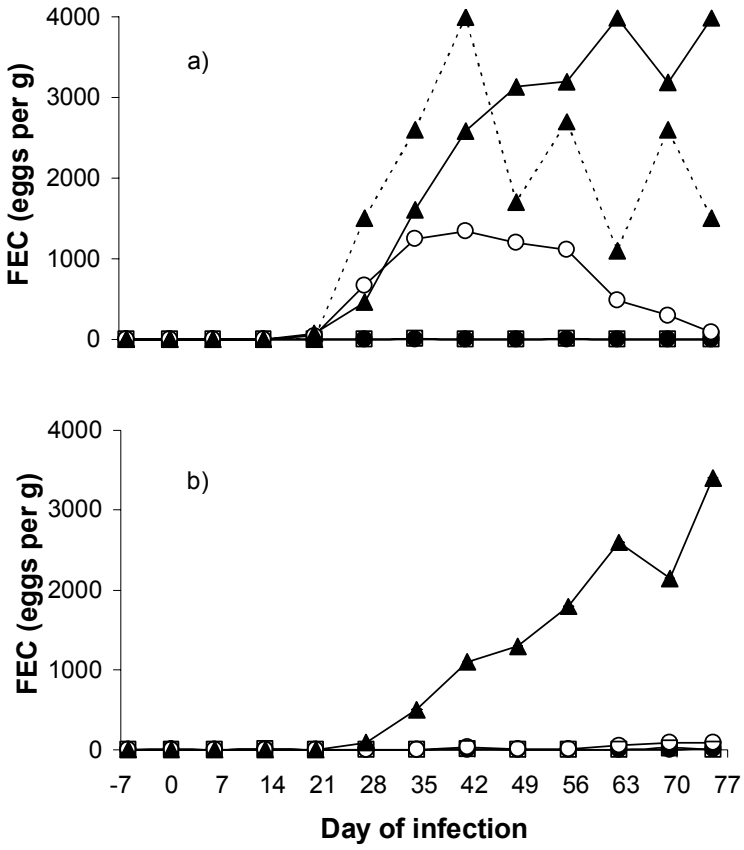

Figure 3.3: Mean back transformed log10 (count + 1) FEC of a) 5-month-old lambs and b) 17-month-old ewes while ○ infected with 2000 or 3200 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS), □ controls (C), and --▲-- animal No55

3.3.4. Worm burdens

Back transformed geometric mean worm burdens for lambs are shown in Table 3.3. Mean worm burdens of 22,387 in ISIF and 224 in IF lambs differed significantly ($p<0.01$). Burdens in both lamb treatments were made up entirely of L5 adult worms.

Back transformed geometric worm burdens for ewes are shown in Table 3.4. Greater numbers of worms were recovered from immuno-suppressed ewes, mean values being 25,632 in ISIF and 160 in IF groups ($p<0.001$). Low numbers of L3 and L4 worms were found in both groups of infected ewes, with adult worms contributing 0.98 and 0.91 of total burden in ISIF and IF groups, respectively. Numbers of L4 worms recovered were low but significantly different between ISIF and IF ewes ($p<0.001$), being 512 and 2 larvae, respectively.

Table 3.3: Numbers of log10 (count + 1) worms recovered from 5-month-old lambs infected with 2000 L3 *T. colubriformis* larvae d^{-1} for 77 days with an intact (IF) or suppressed immunity (ISIF). Back-transformed values are given in parenthesis

	ISIF	IF	s.e.m
L3	0	0	
L4	0	0	
L5	4.35^{a} (22,387)	2.35^{b} (224)	0.46
Total	4.35^{a} (22,387)	2.35^{b} (224)	0.46

[a,b] Values for each stage of larval development with different superscripts are significantly different ($p<0.01$)

Table 3.4: Numbers of log10 (count + 1) worms recovered from 17-month-old ewes infected with 3200 L3 *T. colubriformis* larvae d^{-1} for 77 days with an intact (IF) or suppressed immunity (ISIF). Back-transformed values are given in parenthesis

	ISIF	IF	s.e.m
L3	0.37^{a} (2)	1.00^{a} (10)	0.19
L4	2.71^{a} (512)	0.39^{b} (2)	0.19
L5	4.40^{a} (25,118)	2.17^{b} (148)	0.19
Total	4.41^{a} (25,632)	2.20^{b} (160)	0.26

[a,b] Values for each stage of larval development with different superscripts are significantly different ($p<0.01$)

3.3.5. Worm length

Average worm lengths of adult (L5) *T. colubriformis* are shown in Table 3.5. Overall, worms from IF lambs were relatively 0.14 shorter than worms from ISIF lambs ($p<0.001$) and male worms were proportionately 0.20 shorter than female worms ($p<0.001$). In addition, there was an immuno-suppression x worm sex interaction ($p<0.001$) reflected in female worms from IF animals being comparatively 0.17 shorter than female worms from ISIF animals, while male worms from IF animals were relatively 0.11 shorter than male worms from ISIF animals. For ewes, worms from IF animals were proportionately 0.22 shorter than worms from ISIF animals ($p<0.001$) and male worms were 0.22 shorter than female worms ($p<0.001$). In addition, there was an immuno-suppression x worm sex interaction ($p<0.001$) reflected in female worms from IF animals being comparatively 0.24 shorter than female worms from ISIF animals, while male worms from IF animals were 0.20 shorter than those from ISIF animals.

Table 3.5: Mean length (mm) of worms recovered from 5-month-old lambs and 17-month-old ewes infected with 2000 or 3200 L3 *T. colubriformis* larvae d^{-1} for 77 days with an intact (IF) or suppressed immunity (ISIF).

	5-month-old lambs			17-month-old ewes		
	IF	ISIF	s.e.m	IF	ISIF	s.e.m
Female	6.37^{a}	7.71^{b}	0.14	5.17^{b}	6.82^{a}	0.07
Male	5.33^{a}	5.96^{a}		4.15^{c}	5.22^{b}	

^{a,b,c} Values for each age cohort with different superscripts are significantly different ($p<0.05$)

3.3.6. Carcass composition and wool growth

Computed tomographic estimates of carcass change and estimates of wool growth are shown in Table 3.6. Amongst groups of lambs there was a significant immuno-suppression x infection interaction for the amounts of fat ($p<0.01$), muscle ($p<0.05$) and bone ($p<0.001$) deposited in the carcass. Infection alone (IF lambs) proportionately reduced fat deposition by 0.38 ($p<0.05$) and muscle growth by 0.31 ($p<0.05$). Irrespective of whether infected or not, immuno-suppressed lambs (IS and ISIF) had similar but non-significant relative reductions in fat deposition of 0.06 and 0.09, respectively, ($p>0.05$ for both), but large reductions in muscle deposition of 0.73 and 0.54, respectively ($p<0.05$ in both cases) compared to C lambs.

There was no interaction between immuno-suppression and infection amongst ewe groups for fat ($p>0.05$), muscle ($p>0.05$) or bone ($p>0.05$) deposition. Amongst groups of ewes, carcass fat deposition was increased by immuno-suppression in groups IS and ISIF relative to C by 0.45 ($p<0.05$) and 0.18 ($p>0.05$), respectively, and was reduced by 0.23 ($p<0.05$) by infection alone (IF). Infection superimposed on immuno-suppression (ISIF) reduced fat deposition by 0.19 ($p<0.05$) compared with immuno-suppression alone (IS). Muscle deposition in ewes was proportionately reduced by 0.06 ($p>0.05$) by infection alone (IF), and by 0.70 ($p<0.05$) by immuno-suppression alone (IS). In contrast to the observations in lambs, infection in addition to immuno-suppression in ewes caused a further reduction ($p<0.05$) in muscle deposition (ISIF compared to IS).

There was an immuno-suppression x infection interaction for wool production in lambs ($p<0.05$) but not ewes ($p>0.05$). Relative to C, clean wool production was significantly reduced by 0.16, 0.17 and 0.16 in IF, ISIF and IS lambs, respectively, ($p<0.05$ in all cases). Wool production was not affected by infection in ewes, but was reduced by 0.32 in IS compared to C ewes ($p<0.05$). Infection in addition to immuno-suppression in ewes caused no further reduction in wool production.

Table 3.6: Computer tomographically estimated carcass growth, wool production and energy utilization of 5-month-old lambs and 17-month-old ewes infected with 2000 and 3200 L3 *T. colubriformis* larvae d^{-1}, respectively, (IF), similarly infected and immuno-suppressed (ISIF), immuno-suppressed only (IS), control (C) and No 55.

	5-month-old lambs						17-month-old ewes				
	IF	ISIF	IS	C	No 55	s.e.m	IF	ISIF	IS	C	s.e.m
Original composition (day -14)											
Liveweight (kg)	23.9	24.1	24.2	24.3	23.8	0.64	43.9	44.3	43.0	44.1	1.30
Carcass weight (kg)	11.2	11.3	11.4	11.4	11.2	0.40	23.6	23.9	23.1	23.7	0.80
Bone weight (kg)	1.47	1.47	1.49	1.48	1.46	0.04	2.52	2.54	2.48	2.53	0.06
Fat weight (kg)	1.99	1.88	1.92	1.71	2.20	0.15	7.77	7.36	6.97	7.37	0.41
Muscle weight (kg)	7.86	7.96	8.16	8.25	7.50	0.28	13.3	14.0	13.6	13.8	0.52
Tissue deposition (day 76)											
Bone (kg)	0.67^{a}	0.78^{b}	0.74^{ab}	1.01^{c}	0.49	0.04	0.52^{b}	0.40^{a}	0.62^{b}	0.62^{b}	0.05
Fat (kg)	4.41^{a}	6.46^{b}	6.63^{b}	7.08^{b}	4.12	0.39	3.52^{a}	5.40^{b}	6.66^{c}	4.59^{b}	0.39
Muscle (kg)	2.54^{b}	1.70^{ab}	0.99^{a}	3.68^{c}	0.85	0.43	2.89^{c}	-0.59^{a}	0.92^{b}	3.08^{c}	0.44
Clean wool weight (kg)	1.07^{a}	1.06^{a}	1.08^{a}	1.28^{b}	0.75	0.04	1.27^{b}	0.84^{a}	0.90^{a}	1.33^{b}	0.05
Energy utilization†											
Total ME intake (MJ)	1203^{a}	1510^{b}	1418^{b}	1485^{b}	1172	46	1418	1460	1518	1509	45.2
Total NE deposited (MJ)	225^{a}	304^{b}	308^{b}	347^{c}	195	15	195^{a}	241^{b}	302^{c}	241^{b}	16.4
NE:ME	0.18^{a}	0.20^{ab}	0.21^{bc}	0.23^{c}	0.16	0.01	0.13^{a}	0.16^{b}	0.19^{c}	0.15^{b}	0.01

[a,b,c] Within each age cohort, values within rows with different superscripts are significantly different ($p<0.05$)

† ME = metabolizable energy. NE = net energy

3.3.7. Serum proteins

Serum total protein concentrations were not affected by treatment in either lambs or ewes, mean values being 61.6 ± 1.1 and 65.7 ± 1.2g l^{-1}, respectively. Changes in serum albumin concentrations for lamb and ewe groups are given in Figure 3.4. In lamb groups, there was a significant treatment x time interaction ($p<0.001$). This reflected a significant decline in concentrations in IF lambs during the course of infection, while the remaining groups suffered only a temporary reduction between days 21 and 49. In ewe groups, there was a significant effect of immuno-suppression ($p<0.001$) and time ($p<0.001$), but no interaction between treatment and time ($p>0.05$). Mean serum albumin increased with time and tended to be greatest in immuno-suppressed animals. In contrast to the observations in lamb groups, infected (IF) ewes did not experience a reduction in serum albumin.

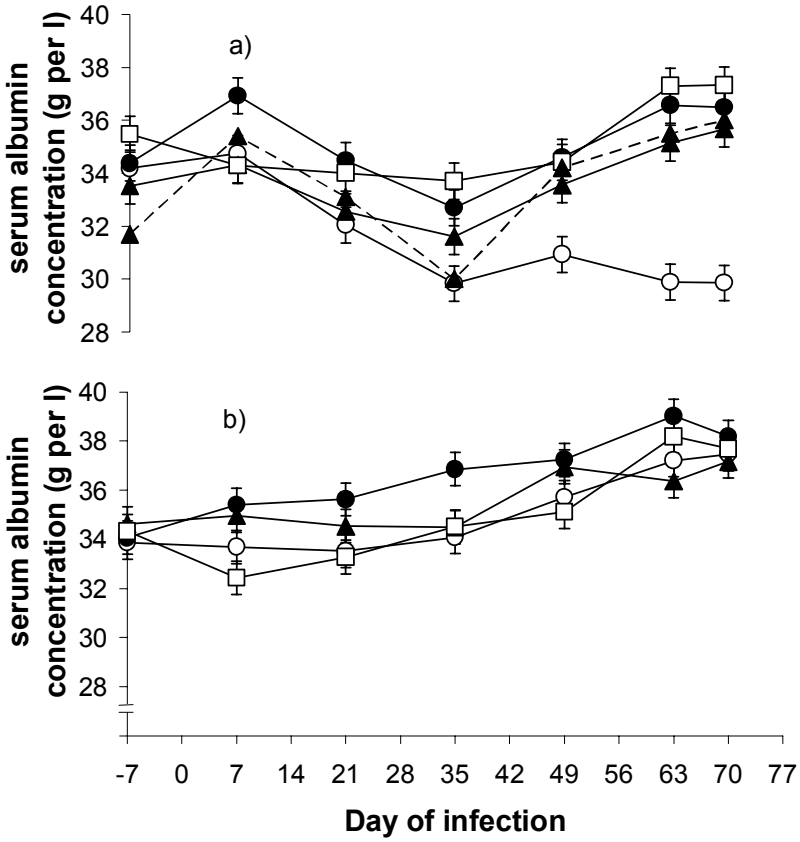

Figure 3.4: Mean serum albumin concentration of a) 5-month-old lambs and b) 17-month-old ewes while ○ infected with 2000 or 3200 *T. colubriformis* d^{-1} (IF), ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS), □ controls (C) and --▲-- animal No55

3.3.8. Serum urea

Changes in serum urea concentrations in lamb and ewe groups are given in Figure 3.5. In lambs, urea concentrations increased in all groups with time ($p<0.001$) and were greater in immuno-suppressed animals ($p<0.001$) with values in ISIF lambs being consistently elevated compared to IF lambs by 0.14 - 0.31 from day 49 ($p<0.05$) and greater than in C lambs on days 49 and 70 ($p<0.05$ on both occasions). In ewe groups, there was a significant effect of time ($p<0.001$), infection ($p<0.05$) and immuno-suppression ($p<0.001$). These effects were reflected in the increase in values with time in all animals, and greater levels in immuno-suppressed animals. Within immuno-suppression treatments, urea values tended to be greater in those exposed to nematode larvae, although this was only significant for ISIF and IF ewes relative to IS and C, respectively, on day 49 ($p<0.05$ in both cases).

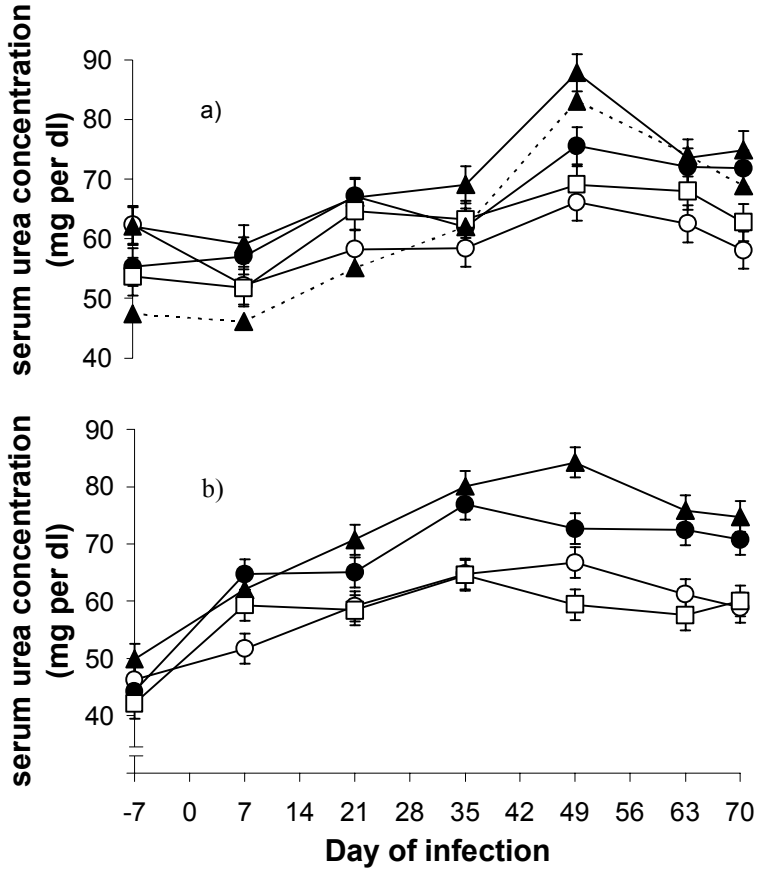

Figure 3.5: Mean serum urea levels of a) 5-month-old lambs and b) 17-month-old ewes while ○ infected with 2000 or 3200 *T. colubriformis* d^{-1} (IF), ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS), □ controls (C) and --▲-- animal No 55

3.3.9. Serum phosphate

Mean serum phosphate concentrations for lambs and ewes are given in Figure 3.6. In lambs, overall there was an infection x time interaction (p=0.01) reflected in a decrease in serum phosphate concentrations in both infected (IF and ISIF) groups from day 21, while levels were maintained in uninfected groups. In ewes, infection tended to reduce serum phosphate (p=0.052) and there was a suppression x time interaction (p=0.006) that was reflected in an increase in concentration with time in both non-suppressed groups but not in immuno-suppressed groups.

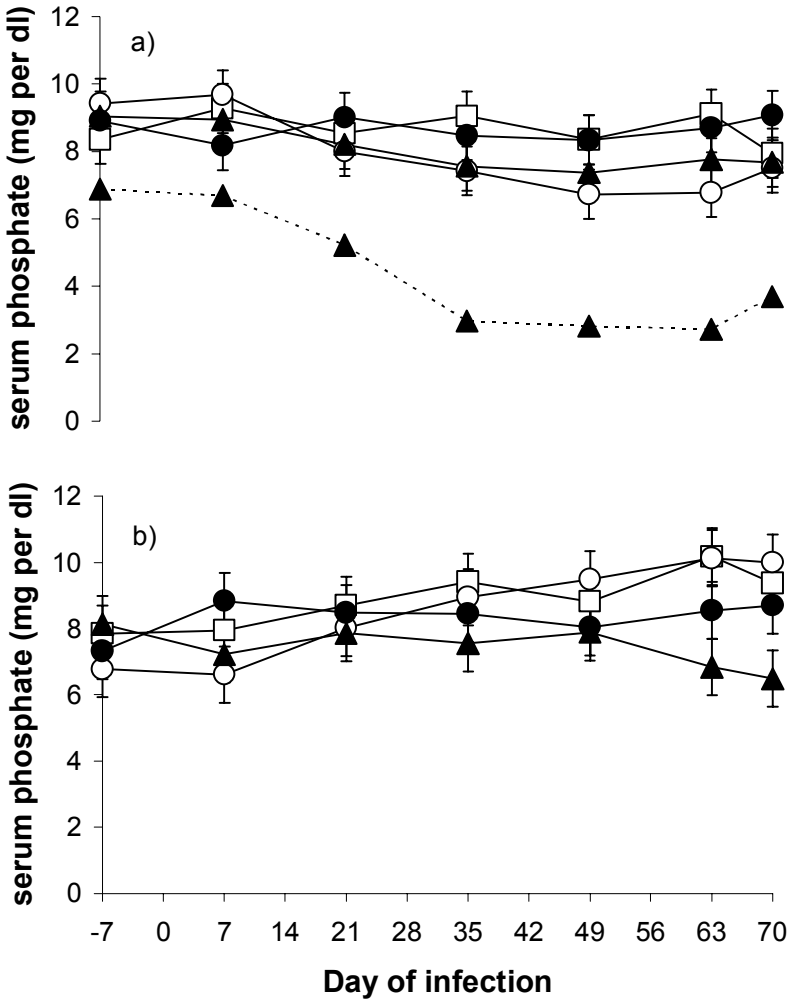

Figure 3.6: Mean serum phosphate concentrations of a) 5-month-old lambs and b) 17-month-old ewes while ○ infected with 2000 or 3200 *T. colubriformis* d^{-1} (IF), ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS), □ controls (C) and --▲-- animal No 55

3.3.10. Serum total antibody

Absorbance of total L3 antibody to *T. colubriformis* in lambs and ewes during infection is given in Figure 3.7. In lambs there was a significant group x time interaction ($p<0.001$). Levels in IF lambs increased gradually from day 21 to a three-fold increase from day 63 ($p<0.001$). Antibody levels in the three remaining groups of lambs were similar and showed no change during the trial. In the ewes, there was a significant treatment x time interaction ($p<0.001$) and an effect of time ($p<0.001$). These reflected the fact that antibody levels declined continuously with time, but at a slower rate in IF and at a greater rate in IS ewes, which were immuno-suppressed without exposure to nematode larvae.

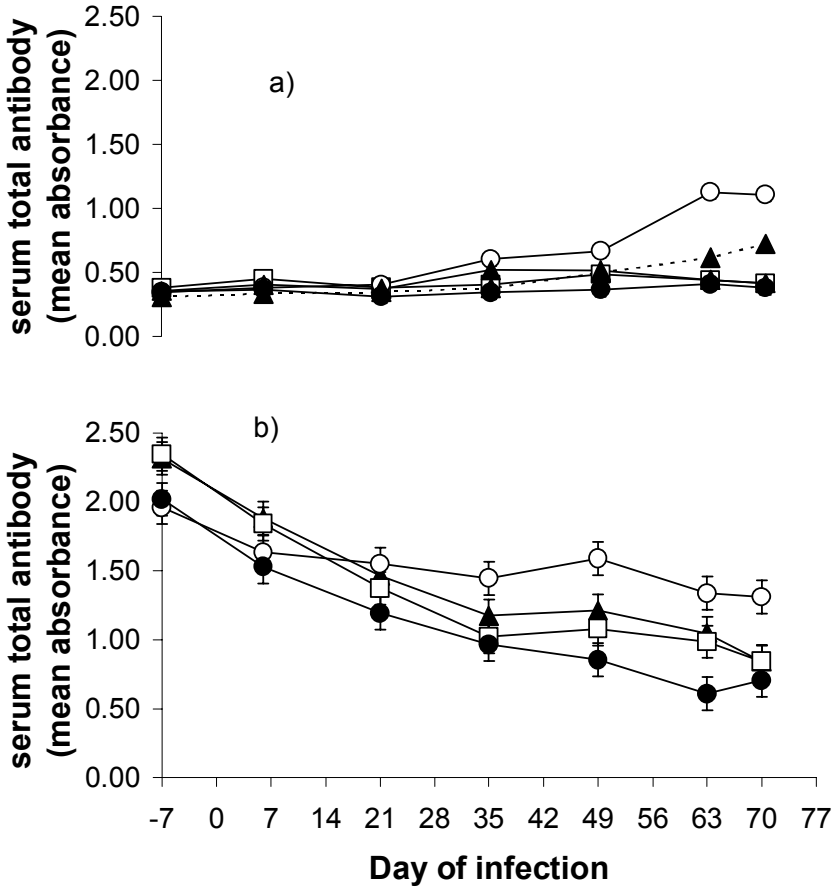

Figure 3.7: Mean total parasite specific L3 *T. colubriformis* antibody levels of a) 5-month-old lambs and b) 17-month-old ewes while ○ infected with 2000 or 3200 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS), □ controls (C), and --▲-- animal No55

3.3.11. Serum L3 IgA

Mean L3 parasite-specific IgA absorbance for lamb and ewe groups is shown in Figure 3.8. There was no treatment x time interaction amongst lamb groups ($p>0.05$). However, there was an effect of infection ($p<0.01$) and time ($p<0.01$). A rise in mean absorbance in IF lambs peaked at approximately three times that of C lambs on day 49 ($p<0.01$) before declining to levels 2.2 times higher at day 63 ($p<0.05$). IgA levels in C, IS and ISIF lambs were similar and showed no change with time. Lamb No 55 expressed an IgA profile similar to that of IF lambs. In all four ewe groups mean IgA absorbance was low throughout the trial, with levels being similar to those in the uninfected lambs. Statistically there was a time x treatment interaction ($p<0.001$). However, there was no consistent pattern between groups and the differences were extremely small, and not considered to be biologically important.

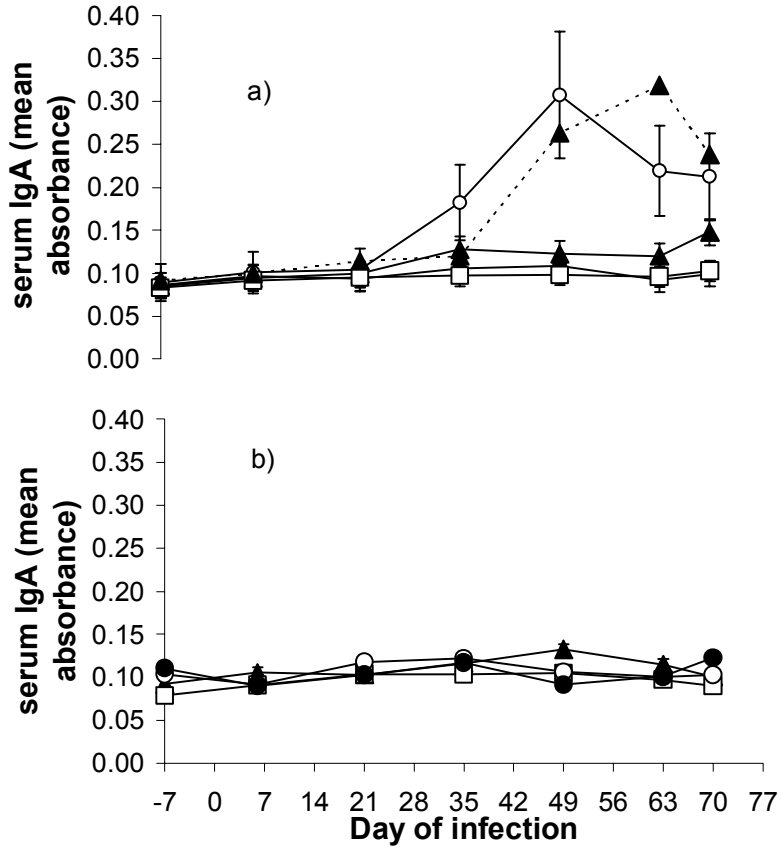

Figure 3.8: Mean parasite specific L3 *T. colubriformis* IgA levels of a) 5-month-old lambs and b) 17-month-old ewes while ○ infected with 2000 or 3200 *T. colubriformis* d^{-1} (IF), ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS), □ controls (C), and --▲-- animal No55

3.4. Discussion

These findings provide strong support for the hypothesis that a significant part of the reduction in animal performance during nematode parasitic infection is a consequence of the host immune response to the parasite rather than simply the effect of damage to the alimentary tract and the cost of its repair, as has been hypothesised in the past (MacRae, 1993; van Houtert and Sykes, 1996). Moreover, the findings disclose the possibility that, in young animals, physiological changes accompanying the acquisition phase of the immune response are implicated in the reduction in appetite that is typical in subclinical infections in young animals with this parasite (Sykes and Coop, 1976; Kyriazakis *et al*. 1996). They also provide novel evidence that the immune response of a mature resistant animal imposes a significant nutritional cost.

The suppression of immunity through the use of corticosteroids was, of necessity, a crude approach, and disturbances to intake, wool growth, liveweight gain, nutrient partitioning and protein metabolism as well as to the immune response were anticipated (Thompson *et al*. 1995; Bertozzi *et al*. 2000), and are discussed later.

The worm burdens of 22,000 and 25,000 (Tables 3.3 and 3.4) in immuno-suppressed (ISIF) lambs and ewes respectively, were much lower than the 267,000 found after immuno-suppression of 15 month old hoggets by Nagasinha (1999), and low even compared to the 45-51,000 found by Sykes and Coop (1976) and Bown *et al.* (1991a) using similar rates of infection with this nematode in naïve but immunologically normal lambs. The larvae used in the current study can therefore be considered, on this basis, to have had low infectivity. Evidence for effective immuno-suppression in the current study can be observed in the much larger worm burden, greater FEC (Figure 3.3), and longer worms (Table 3.5) in ISIF animals compared with their IF counterparts, and can be confirmed by the apparent lack of antibody production in immuno-suppressed animals (Figures 3.7 and 3.8). On the other hand, the 0.30

proportionate depression in live weight gain of the infected but non-immuno-suppressed lambs (IF) was comparable with the typical 0.18 – 0.60 reductions observed by Sykes and Coop (1976) and Bown *et al*. (1991a) in naïve lambs exposed to similar rates of infection with this parasite, suggesting 'normal' pathogenicity.

The reduction in wool growth in immuno-suppressed lambs and ewes (IS) (Table 3.6) was expected since glucocorticoid analogues are known to reduce wool production by up to 0.80 and cause wool breaks that may result in defleecing (Panaretto 1979). Perhaps surprisingly, therefore, no obvious signs of wool break were observed in any of the immuno-suppressed animals. Gastro-intestinal nematode infections are known to disturb wool growth (MacRae, 1993). However, larval challenge in ewe groups with a competent immune response did not reduce wool production, findings that contrast with the 0.11 relative reductions in wool growth in animals resistant to *T. colubriformis* observed by Barger and Southcott (1975). The 0.16 reduction in wool growth in the IF lambs is in agreement with observations in lambs exposed to similar rates of infection by Sykes and Coop (1976). This, together with a lack of effect in immunologically competent ewes suggests competition for potentially rate limiting amino acids for wool production, probably the sulphur amino acids (MacRae, 1993), may be greatest during the phase of acquisition of immunity. Further evidence to support this hypothesis can be observed in data from breeding ewes in which established immunity is temporarily lost in the period around parturition. Leyva *et al*. (1982) infected groups of sheep with *Ostertagia circumcincta* during the last six weeks of pregnancy and the first six weeks of lactation. These authors observed significant reduction in wool growth only during the latter period, as immunity was being re-established following its relaxation around parturition.

Regardless of infection status, live weight gain was depressed in young animals treated with the corticosteroid (Figure 3.2). While some of this reduction in growth rate of the IS group of lambs can be attributed to the 0.03 proportionate reduction in feed intake, a major cause was the greater than 0.50 reduction in muscle (protein) deposition as a result of immuno-suppression treatment (Table 3.6), presumably as a consequence of the net catabolic actions of corticosteroid compounds (Huang *et al*. 1998; Turini *et al*. 2003). It was perhaps surprising, therefore, that a greater than 0.03 reduction in feed intake did not occur in IS lambs since demand for energy for protein synthesis has been considered to be a major driver of appetite (Radcliffe and Webster, 1976). The situation created by corticosteroid treatment, therefore, seems to have been one of reduced net muscle protein deposition, presumably resulting in an increased amino acid supply to support the synthesis of constitutive and export proteins in the liver, including acute phase proteins for the immune system (Husband and Bryden, 1996; Calder and Newsholme, 2002). Turini *et al*. (2003) observed in rats treated with dexamethasone that protein synthesis rates were reduced in muscle and increased in the liver, resulting in an increase in liver weight. Evidence for such an effect in the current trial can be seen in the absence of the typical depression in serum albumin (Figure 3.4) and correspondingly greater liver weights of ISIF lambs, and a similar trend in liver weights in ISIF ewes compared to their IF counterparts. The relatively high rate of adipose tissue deposition in corticosteroid-treated animals (Table 3.6) has been observed previously in cattle (Dicke *et al*. 1974; Corah *et al*. 1995), and probably reflects an increased availability of deaminated carbon skeletons, as evidenced in elevated serum urea concentrations in immuno-suppressed groups (Figure 3.5), and presumably low demand for metabolizable energy (ME) for body protein synthesis.

Immuno-suppression appeared to reduce the energetic costs associated with nematode infection. The lowest rate of fat deposition occurred in both age cohorts of infected (IF) sheep (Table 3.6). Such changes have been interpreted to reflect the energy cost of increased protein synthesis, presumably for the repair of damaged tissue in the alimentary tract (van Houtert and Sykes, 1996; Yu *et al.* 2000). It is interesting, therefore, that animals from both age cohorts, when concurrently infected and immuno-suppressed (ISIF), had rates of adipose tissue deposition that were similar to those of their immuno-suppressed controls (IS) but greater than those of groups simply infected (IF). This suggests that very little energy was invested in the repair of gastro-intestinal tissue in infected immuno-suppressed animals (ISIF). It seems unlikely this can be attributed to differences in ME supply, as the intake of the older (ewe) groups did not differ and there was no measurable effect of immuno-suppression on dry matter (DM) digestibility, nor does the literature suggest DM digestibility is likely to be significantly affected by nematode infection (Sykes and Coop, 1976; Bown *et al.*, 1991b). Corticosteroids have been shown to cause a reduction in the fractional synthetic rate of total protein in the gut mucosa in non-parasitised rats (Turini *et al*. 2003) which may prevent the restoration of damaged tissue. However, it remains a point of interest that ISIF animals were able to harbour a larger parasite burden than their IF counterparts without displaying any clinical signs of parasitism or suffering a reduction in performance or decline in serum albumin typical of this type of infection. Furthermore, a comparable reduction in the serum phosphate concentration of ISIF and IF lambs from day 21 (Figure 3.6) suggests a similar reduction in phosphorous absorption as a consequence of intestinal pathology. It can be hypothesised, therefore, that the parasite itself caused little direct damage to the intestinal endothelium in ISIF animals. Hein *et al*. (2001) have suggested many of the pathological changes in the gastrointestinal tract may be undesirable consequences of the immune response. This is supported by the findings of Lawrence *et al*. (2001), in which mast cell deficient mice had longer intestinal villi than their immunologically competent counterparts after 13 days of

infection with *T. spiralis*, despite harbouring a greater worm burden. It was hypothesised by Lawrence *et al*. (2001) that one of the roles of the immune response was to act directly on the gastrointestinal tissue, making the environment unfavourable for the parasite and, as a consequence, becoming the major cause of intestinal pathology.

The experimental design allowed the comparison of the energetic costs of nematode infection during the development of immunity in young naïve animals (IF lambs) with those incurred by older animals (IF ewes) capable of maintaining a competent immune response as judged by their ability to limit FEC during larval challenge. The gross efficiency of utilization of ME for growth was calculated as the proportion of total ME intake that was deposited as net energy (NE) in the carcass and fleece (Table 3.6). Infection alone caused a proportionate reduction in the gross efficiency of utilization of ME of 0.20 and 0.16 in IF lambs and ewes, respectively. The reduction in efficiency observed in the lambs is low in comparison to the 0.50 reduction observed in growing lambs with similar rates of infection with this nematode by Sykes and Coop (1976). There are no precise data for the effect of infection on efficiency of ME use in animals with a competent immune response. However, the 0.16 reduction recorded in IF ewes suggest a significant loss in productivity as a consequence of maintaining an established immunity, a figure comparable to the estimated 0.15 increase in maintenance requirement suggested by Sykes (1994).

Reduction of food intake during nematode infections of young sheep can vary from less than 0.10 to complete anorexia, and has been calculated to be responsible for 0.60 – 0.90 of the loss in performance during nematode infection (Coop and Holmes, 1996; van Houtert and Sykes, 1996), making it the single largest cause of reduced productivity in nematode-infected sheep. Maintenance of an effective immune response in IF ewes was associated with a non-significant 0.06 reduction in overall intake. There are few reliable data on the effect of parasitic infection on the food intake of immune animals, however

intake has often been observed to recover once naïve animals have developed immunity (Kyriazakis *et al*. 1996; Kyriazakis *et al*., 1998). The reduction in intake exhibited by IF lambs was temporary (Figure 3.1), occurring only during the phase of acquisition of immunity, between days 14 and 64 of infection. This reduction and subsequent recovery was typical, both temporally and in magnitude, to comparable subclinical infections with this parasite (Kimambo *et al*. 1988; Kyriazakis *et al*. 1996). Surprisingly, neither of the immuno-suppressed and infected groups (ISIF lambs and ewes) suffered a reduction in appetite, despite faecal egg counts approaching 4000 epg by the end of the trial (Figure 3.3). The effects of corticosteroids on intake in sheep are not well documented, and appear to vary depending on the dose given. Adams and Sanders (1992) found a short term increase in intake in sheep treated with a single dose of 0.1mg kg^{-1} dexamethasone (equivalent to 0.5mg kg^{-1} methylprednisolone). On the other hand, Panaretto (1979) observed a short term decrease in intake in sheep treated with a larger single dose of 6mg kg^{-1} dexamethasone. There was no effect of a weekly chronic dose of 1.3mg kg^{-1} methylprednisolone acetate on intake in the current study as at all times both age groups of IS animals consumed food at the same rates as their respective C groups. The results from the current trial provide strong evidence that the greatest challenge to maintenance of feed intake during nematode larval challenge occurs during the phase of acquisition of immunity in the naïve lamb. Kyriazakis *et al*. (1998) suggested that anorexia during parasitic infection may be viewed as a disease coping strategy that aids the recognition of the parasite by the immune system, and that strategies to complement it should be considered to allow greater resilience to the effects of infection. While it is still undetermined if anorexia does promote a beneficial immune response, the absence of both anorexia and loss of performance in ISIF lambs suggests a depression in feeding behaviour during nematode infection is not essential for the animal to express greater resilience to cope with infection.

The loss of appetite in IF lambs appeared to coincide with the period of elevation of serum IgA antibodies against *T. colubriformis* L3 larvae. Furthermore, feed intake of the one lamb from group ISIF (No 55) which, on the basis of a comparable change in serum IgA to IF lambs (Figure 3.8) could be considered not to have responded to immuno-suppression, was depressed in a similar pattern to that of IF lambs. Moreover, in the ewes which did not show significant reductions in appetite no elevation of IgA was observed. These data suggest that physiological changes associated specifically with the acquisition phase of the developing immune response and which are associated with the stimulation of the IgA antibody response, rather than the mature immune response *per se*, are responsible for the loss of appetite in infected sheep. A similar relationship between food intake and a component of the immune response was observed in infected lambs by Kimambo *et al*. (1988), whereby elevated eosinophil concentrations during the acquisition phase of the immune response corresponded with the period of maximum reduction in voluntary intake. Furthermore, these authors observed that once immunity was successfully achieved, as judged by faecal egg counts, a decline in eosinophil concentrations was accompanied by an increase in voluntary feed intake. Pro-inflammatory cytokines involved in the signalling of the non-specific acute phase response (APR) are considered to act on the hypothalamus through the afferent pathway to cause anorexia, lethargy and thermogenesis in human and murine subjects (Johnson, 1997). While there was no detectable increase in rectal temperature in IF animals associated with anorexia, recent reviews have identified IFNγ, TNFα, IL-1 IL-6 and IL-8 as appetite depressing cytokines (Langhans, 2000; Farthing and Ballinger, 2001). It is suggested therefore, that the lack of effect on appetite in the immuno-suppressed young lambs resulted from their inability to promote an acute phase cytokine response. In addition, the lack of reduction in appetite in mature ewes reflects the fact that their immune response had evolved to a specific immunity and was therefore less dependent on an APR and the consequences of its cytokine profile.

The evidence suggesting the immune response *per se* may have detrimental components substantiates reservations raised by Colditz (2002) concerning the benefits of strong immune responses to nematode infection in young lambs. Genetic selection of sheep for low immune responsiveness (high FEC) has resulted in greater productivity in terms of wool growth and liveweight gain, whereas lines selected for low FEC have shown lower growth rates than both their random bred controls and high FEC lines (Howse *et al.* 1992; Morris *et al*. 2001). Pernanther *et al.* (1997) found *T. colubriformis* specific antigen stimulation of mesenteric lymph node cells taken from genetically resistant lambs promoted an almost 4-fold greater increase in the pro-inflammatory cytokine IFN-γ than cells taken from susceptible lambs. It remains unclear if the reduction in performance in lines selected for a strong immune response is due to greater partitioning of nutrients to immune function as suggested by Coop and Kyriazakis (1999), or if a strong developing immune response has physiological consequences that result in a more severe depression of appetite and consequent reduction in performance during the acquisition phase of immunity.

The implications of this work are that a component of the developing immune response, possibly involving pro-inflammatory cytokines, may be responsible for the reduction in voluntary feed intake in parasitised lambs. This provides a conflicting situation in which productivity is severely reduced through a reduction in intake during the acquisition phase of immunity but is little affected once an effective immune response is established. The challenge is to identify the exact mechanisms involved and develop strategies that assist the animal in achieving a mature immune status at minimum nutritional cost, or facilitate a rapid shift from the phase of development to the mature immune response.

Chapter 4

The influence of metabolizable protein supply on the metabolic disruption caused by the immune response in lambs during infection with *Trichostrongylus colubriformis*

4.1. Introduction

The inevitable infection of young grazing lambs with gastro-intestinal nematode parasites causes a considerable metabolic and nutritional disturbance. This disturbance is reflected in a reduction of nutrient utilization as a consequence of increased demand for energy and/or protein for the development and maintenance of immunological function coupled with reduced voluntary feed intake. Of particular importance is the temporary reduction in feed intake that can range in severity from as little as 0.10 through to complete anorexia, and has been attributed to causing 0.60-0.90 of production losses in infected lambs (van Houtert and Sykes, 1996). Not surprisingly, the cause of reduced appetite has received considerable attention (Dynes *et al*., 1990; Kyriazakis *et al*., 1998). Corticosteroid-induced immuno-suppression of sheep (described in Chapter 3) provided evidence that components of the animals' developing immune response may be involved in the reduction of feed intake during infection. Although the detrimental components of the developing ovine immune response have not been identified, pro-inflammatory cytokines involved in the predominantly Th-1 type immunological cascade have been shown to have anorectic properties in mice and humans (Johnson, 1998), the production of which is suppressed by cytokines involved in a Th-2-type immunological reaction (Jankovic *et al*., 2001). As immune development is governed primarily through cytokine expression, theoretically, manipulations that advance the development of immunity must also be considered to have the potential to influence immunological signalling. Development of immunity to gastro-intestinal parasites in young lambs has been shown to be advanced

through the provision of additional protein (Bown *et al*., 1991a; Coop *et al*., 1995) presumably through the supply of amino acids for the production of mast cells, immunoglobulins and lymphokines involved in the effecter mechanism of parasite expulsion that are proteinacous in nature (Kambara *et al*., 1993). In addition, animals offered high protein diets appear to have a reduced metabolic disturbance as a consequence of infection compared to their low protein fed counterparts (Kyriazakis *et al*, 1994; van Houtert *et al*., 1995). Protein supply has been shown to alter cytokine signalling in mice (Ing *et al*., 2000), therefore, it is plausible that protein supplementation in parasitised lambs results in changes to the immunological signalling or the response to that signalling from a predominantly metabolically disruptive pro-inflammatory reaction to a protective immunity that is effective at expelling the nematode burden. As elucidation of this would require the measurement of cytokine responses that are not currently available, the aim of this trial was, therefore, to examine the hypothesis that additional protein supply in nematode infected lambs influences the consequence that immunological signalling has on feed intake and the metabolic cost of infection in young lambs.

4.2. Materials and Methods

4.2.1. Animals and treatments

Fifty-two mixed sex Coopworth lambs were contrived to have minimal nematode larval experience until the start of the trial at 5 months of age as a consequence of weaning and housing at six weeks of age. Twenty four were from dams that were grazing pasture and twenty eight were from dams that had been housed for other experimental purposes. All lambs from the former treatment group were drenched with 1ml 5 kg^{-1}liveweight (LW) of a combination drench (37.5g l^{-1} levamisole and 23.8g l^{-1} albendazole, Arrest, Ancare New Zealand Ltd, Auckland, New Zealand) when removed from pasture. Lambs were allocated into one of eight groups that were balanced for LW, liveweight gain from housing, animal origin (housed or pasture) and sex and had a mean LW of 27.6 ± 1.8 kg. Four groups were offered a low protein (L) and four a high protein (H) diet. Within each dietary treatment, one group was infected with the equivalent of 2000 L3 *Trichostrongylus colubriformis* larvae d^{-1} (IF; n=8), a second group (ISIF; n=8) received the same infection but with immune function suppressed by weekly intramuscular injection of the glucocorticoid methylprednisolone acetate, (Depo-Medrol, 40mg methylprednisolone acetate ml^{-1}, The UpJohn Company, Kalamazoo, U.S.A) at a rate of 1ml 30$kgLW^{-1}$. A third group received only the glucocorticoid (IS; n=4) and the fourth remained as a control (C; n=6). An additional eight male hogget rams were housed in metabolism crates to determine digestibility of the L and H diets offered, using methods described in Chapter 3.

4.2.1. Feeding and sampling

Animals were offered fresh water and one of two complete ruminant rations *ad libitum* daily, designed to supply high or low levels of metabolizable protein (MP). The composition of each diet is given in Table 4.1. Individual feed refusals were collected and weighed weekly. Sub-samples of feed offered and refused were taken for determination of dry matter (DM) after drying for 72h at 90^{o}C. Liveweight was recorded at weekly intervals. Fasted LW (after 24h of

food deprivation) was also measured on days 1 and 77 of infection to enable the estimation of body composition by computer tomography (described below). Blood samples were taken weekly from day 1 using jugular venipuncture into a 10ml vacutube (Becton Dickinson, VACUTAINER Systems, Rutherford, New Jersey, U.S.A) and stored at 4°C for 24h. After centrifugation at 2500rpm for 10min serum was separated and stored at –20°C.

Table 4.1: Diet composition (g $kgDM^{-1}$) and analysis for the high protein (H) and low protein (L) rations which were offered *ad libitum*

	High Protein	Low Protein
Fish meal	97	-
Molasses	50	50
Barley	369	600
Lucerne Chaff	348	76
Oat Hulls	116	250
Sodium Phosphate	4	4
Dicalcium Phosphate	5	7
Potassium Carbonate	8	8.8
Magnesium Oxide	0.6	0.7
Potassium Chloride	1	1.5
Mineral Mix†	1	1
Analysis		
CP (g $kgDM^{-1}$)	175	93
MJME $kgDM^{-1}$	10.5	11.1
Crude Fibre	226	167
MP supply (g $kgDM^{-1}$)	95	62
DTUP5‡	31	8
RP5§	120	84

NB. CP = crude protein, MJME = mega joules of metabolizable energy
MP = metabolizable protein
† 1kg contains: 5MIU vit A, 1MIU vit D, 7500IU vit E, 1g Co, 1g I, 20g Mn, 26g Fe, 25g Zn, 210g Mg, 4g P, 0·25g Se, 560g Ca
‡ Estimated digestible true undegraded protein at 5% rumen outflow rate (Agriculture and Food Research Council (AFRC), 1993)
§ Estimated rumen degradable protein at 5% rumen outflow rate (AFRC, 1993)

4.2.2. Parasitology and slaughter/necropsy procedure

Groups IF and ISIF of both feeding regimes were infected with the equivalent of 2000 L3 *T. colubriformis* larvae d^{-1} (80 L3 larvae kg initial LW^{-1} d^{-1}) in three doses each week from day 1 until day 73 using the technique described in Chapter 3. Weekly faecal samples were taken directly from the rectum for the determination of faecal nematode egg concentration (FEC; eggs $gram^{-1}$ (epg)) as described in Chapter 3. The remainder of the faecal sample not required for FEC was used to estimate faecal DM % from day 42 after drying for 72 h at 90°C. Infected lambs were fasted for 24h before slaughter on day 77. Slaughter of infected animals, worm recovery, counting and worm length measurements were as described in Chapter 3, and carcass weight (CW) and liver weights were recorded.

4.2.3. Serum analysis

Total antibody and immunoglobulin A (IgA) specific to *T. colubriformis* L3 larvae in the serum were measured using an enzyme-linked immunosorbent assay (ELISA) as described in Chapter 3, with the exception that colour was developed for 15 and 40 min for total antibody and IgA, respectively. Serum urea, phosphate, total protein and albumin concentrations were determined using a Cobas Mira Plus Auto-analyser (Roche Diagnostics GmbH, Mannheim, Germany) as described in Chapter 3, with serum globulin levels calculated by difference.

4.2.4. Body composition

Changes in the bone, muscle and fat content of the carcass were estimated *in vivo* using x-ray computed tomography on days –8 and 76 of infection. Sedation, restraint and scanning procedure were as described in Chapter 3. Briefly, three anatomical reference x-ray cross sections were taken from each animal at the thoracic vertebrae 8, lumbar vertebrae 5 and ischium. In addition, six animals were randomly selected on each occasion for estimation of total carcass tissue weight using the calvaleri principle of Gundersen and Jensen (1987). Calvaleri-estimated CW was corrected to actual CW obtained from slaughtered animals. Carcass composition for the animals that did not undergo the calvaleri scanning procedure was estimated from the three references slices as described in Chapter 3 with the following exceptions: CW for all animals at both scanning times were estimated using the formula: *CW (kg) = (0.5091 X fasted LW) – 0.9005* (R^2=0.95); *calvaleri fat % = (1.0295 X fat % in the reference slices) – 0.2989* (R^2=0.99); *calvaleri muscle % = (0.9743 X muscle % in the reference slices) + 0.4211* (R^2=0.95). The net energy (NE) deposited in the carcass gain was calculated assuming muscle tissue consisted of 0.20 protein using energy values of 38.9 and 22.2 mega joules (MJ)kg^{-1} for fat and protein, respectively (Blaxter and Rook, 1953).

4.2.5. Wool production

Animals were shorn on days –5 and 77 of infection and greasy fleece weight recorded on the latter date used as a measure of total wool production. A sub-sample of mid-side fleece wool was taken at shearing from each animal on day 77 and scoured for estimation of clean fleece weight by repeated plunging in water at 60^{o}C containing 1ml l^{-1} Teric GN-9 (ICI, Australia) before washing in clean water and drying at 60^{o}C in a forced air oven. Energy deposition in the fleece was calculated assuming 23.7MJ kg^{-1} clean fleece weight (AFRC, 1993).

4.2.6. Statistical analysis

Data were analysed using GENSTAT statistical package (Lawes Agricultural Trust, 2003). All values are group means unless otherwise stated. During all analyses factors were diet (H or L) and treatment (C, IF, IS or ISIF) with estimates of missing values. Faecal egg counts and worm burdens were log transformed (log10 (count + 1)) before analysis. Worm burden, worm length, wool production, digestibility and carcass composition were analysed by ANOVA. All remaining measurements underwent sequential comparison of ante-dependence structures for repeated measures before being analysed by Restricted Maximum Likelihood (REML) with time included as a factor and standard error of the differences calculated, which were then utilized to compare effects using a one tailed t test.

This experiment was carried out with approval from, and in accordance with the Lincoln University Animal Ethics Committee: Authority LU25/02.

4.3. Results

Two animals from the LIF group suffered from severe anorexia, weight loss and became non-responsive to external stimuli and were consequently euthanased on days 31 and 73, respectively. One LIS animal died on day 37 from suspected blood poisoning, while one from the HIS died of pleurisy in the apical and cardiac lobes of the lung on day 57. One LISIF animal died on day 76 shortly after CT scanning. Although the exact cause of death could not be determined by autopsy, it was found to have hepatization of the right apical and half of the cardiac lobes of the lung in addition to peritonitis in the gut cavity. Data from the dead animals were included in the statistical analyses using estimates of missing values. One LISIF individual (No 20) suffered a severe reduction in appetite after four weeks of infection, with daily measurements of food intake demonstrating only short-term increases in intake for several days after steroid administration. Consequently, this animal received two additional steroid doses on days 41 and 48, thereafter intake returned to acceptable levels. Data from this animal are included in the statistical analysis.

Dry matter digestibility was not affected by corticosteroid treatment, average values being 0.59±0.06, 0.61±0.06, 0.59±0.01 and 0.59±0.08 for HIS, HC, LIS and LC animals, respectively.

Mean liver weights at slaughter were 760 ± 101, 954 ± 146, 495 ± 166 and 655 ± 150 for HIF, HISIF, LIF and LISIF, respectively. Overall, there was a 0.29 proportional increase in liver weight as a consequence of immuno-suppression ($p=0.001$). In addition, animals on high protein diets displayed a 0.33 proportional heavier liver weight than those on low protein diets ($p<0.001$).

4.3.1. Faecal dry matter

Faecal dry matter (DM) percentage from day 42 is given in Figure 4.1. There was a treatment (infection and/or immuno-suppression) x time interaction ($p<0.01$) as a consequence of 0.14-0.35 and 0.16-0.25 relative decreases in faecal DM from days 56 and 63 for HIF and LIF animals compared to HC and LC, respectively. There were no such effects in HISIF or LISIF groups.

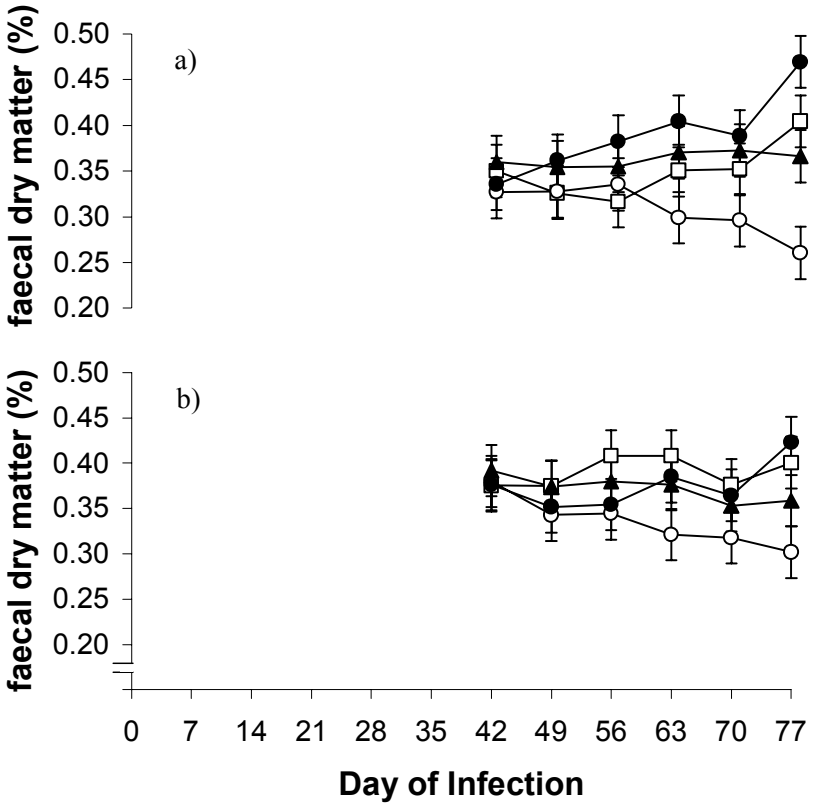

Figure 4.1 Mean dry matter percentage of faeces from lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.3.2. Feed intake

Mean DM intakes are shown in Figure 4.2. There was an effect of diet ($p<0.01$), treatment ($p=0.01$) and time ($p<0.01$) on DM intake. This was reflected in LC animals maintaining a 0.31 to 0.51 proportionately lower mean intake than HC animals throughout the trial while infection alone reduced intake by 0.12 and 0.23 between days 22 and 63 in HIF and LIF sheep, respectively. Immuno-suppression alone had no effect on intake in either L or H groups ($p>0.05$). Within immuno-suppressed groups infection caused no disruption of intake. However, HISIF animals did experience a relative reduction in intake of 0.24-0.27 compared with HC animals from day 63 ($p<0.01$).

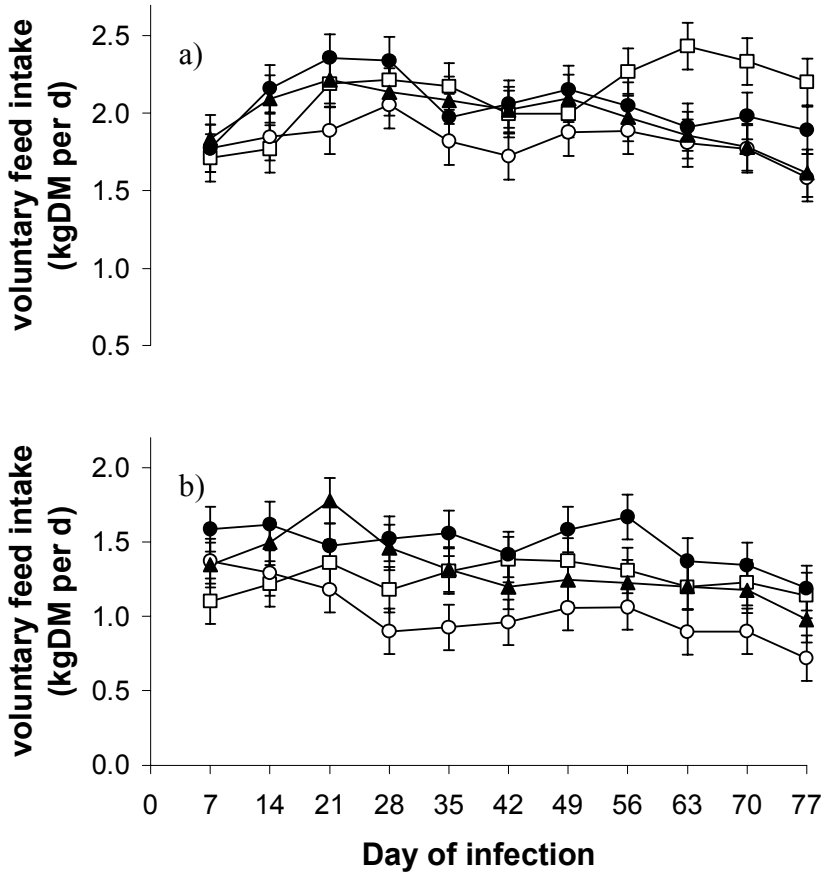

Figure 4.2: Mean voluntary feed intakes of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.3.3. Liveweight

Mean liveweights are shown in Figure 4.3. There were diet x time ($p<0.001$) and treatment x time interactions ($p=0.002$). The former was reflected in an increase in all liveweights with time ($p=0.001$) that was greater in high protein animals. Infection proportionately reduced fasted liveweight on day 77 by 0.18 in HIF ($p<0.001$) and by 0.25 in LIF ($p<0.001$) compared with HC and LC, respectively. Regardless of dietary treatment, immuno-suppression alone had no effect on weight gain. Concurrent infection and immuno-suppression tended to reduce fasted liveweight on day 77 in both high and low protein groups by 0.09 for HISIF ($p=0.06$) and by 0.13 for LISIF ($p=0.09$) compared to HIS and LIS, respectively.

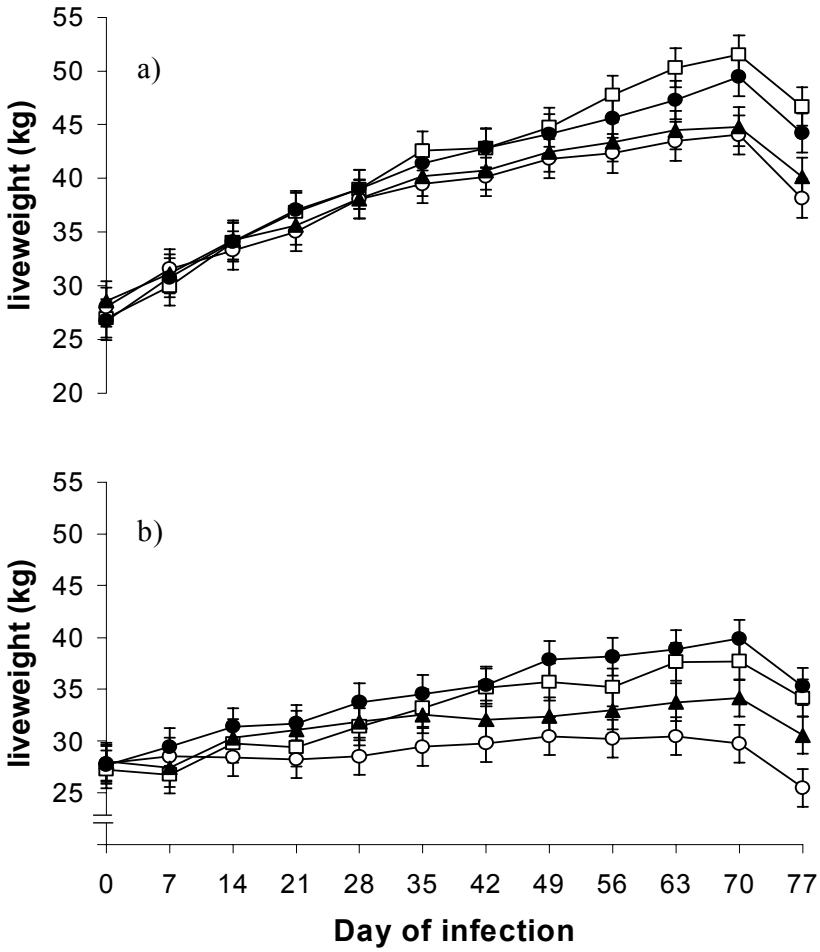

Figure 4.3: Mean liveweight of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C). Values for days 1 and 77 are 24h fasted liveweight.

4.3.4. Faecal egg counts

Nematode eggs in the faeces are shown in Figure 4.4. There was a diet x treatment x time interaction ($p<0.01$) reflected in a higher concentration of eggs in faeces from low protein animals that was not influenced by immuno-suppression, but immuno-suppression of high protein individuals resulting in a greater egg concentration in their faeces. HIF displayed a typical pattern of FEC, with values increasing to a peak of 1900 epg at day 49, thereafter declining to less than 100 epg from day 70. In HISIF sheep FEC continued to rise, reaching 5,400 epg by day 77. In LIF and LISIF animals, FEC increased throughout the trial, reaching peak concentrations at day 77 of 13,800 and 12,100 epg, respectively.

The average concentration of eggs found in all groups was heavily influenced by voluntary feed intake and faecal DM percentage. Consequently, total daily egg production was calculated. However, since faecal DM was only measured from day 42, the faecal DM % for days 21 to 35 was estimated from the measurements on day 42. Back-transformed total 24h faecal nematode egg excretion is shown in Figure 4.5. Overall, there was a highly significant diet x treatment x time interaction ($p<0.001$) reflecting an increase in total egg excretion to a plateau of 1,200,000 eggs d^{-1} by day 42 in both ISIF groups and 400,000 to 700,000 eggs d^{-1} in both IF groups that was maintained in all but HIF, in which counts declined from day 56 to less than 100,000 eggs d^{-1} by day 70.

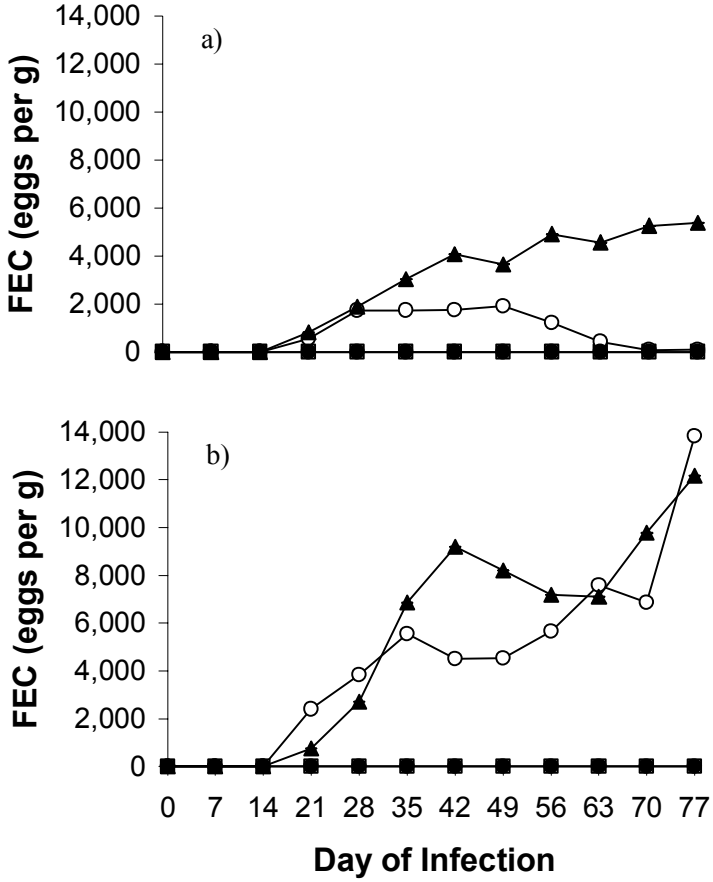

Figure 4.4: Mean back-transformed (log10 (count + 1)) faecal egg count (FEC) of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

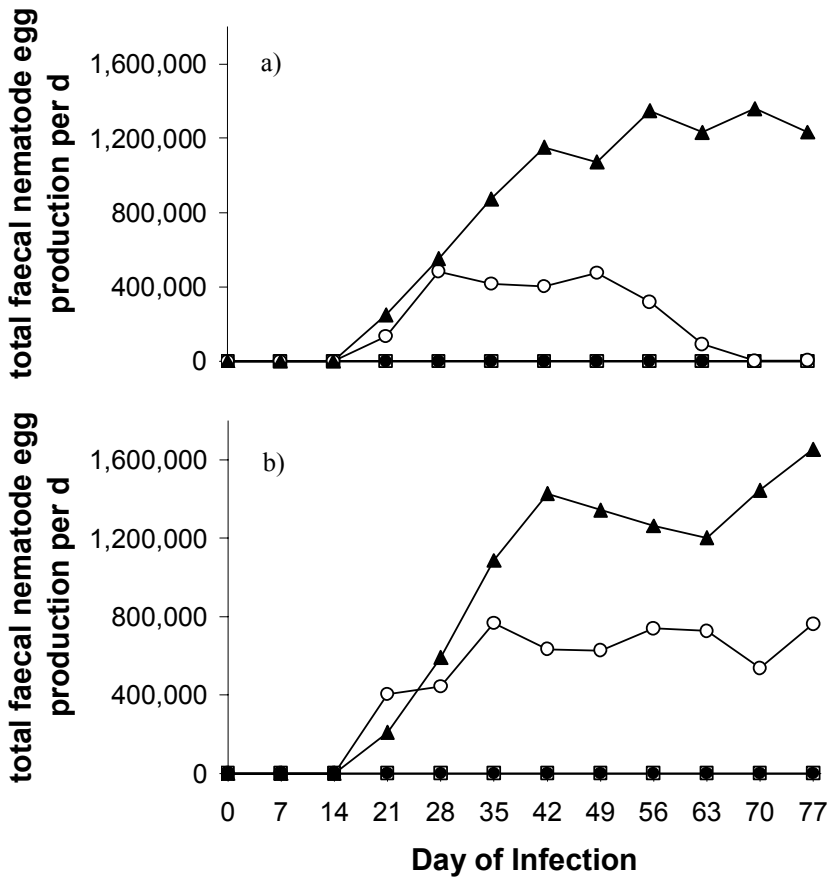

Figure 4.5 Mean back-transformed (log10 (count + 1)) total nematode egg production d^{-1} of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.3.5. Worm burden at slaughter

Worm burdens of infected groups at slaughter are given in Table 4.2. Burdens of all groups consisted proportionately of 0.98-0.99 adult nematodes. Overall, there was an immuno-suppression x diet interaction ($p<0.001$) with immuno-suppression resulting in greater worm burdens in high protein, but not in low protein fed animals compared to their simply infected contemporaries. Across all larval development stages, LIF animals had greater numbers than HIF ($p<0.03$), whereas no effect of protein was observed in immuno-suppressed

animals (p>0.05). The proportion of adults that were female was 0.67 ± 0.11, 0.62 ± 0.06, 0.61 ± 0.05 and 0.59 ± 0.06 for HIF, HISIF, LIF and LISIF, respectively, and was not affected by immuno-suppression (p=0.20) or diet (p=0.09).

Table 4.2: Numbers of log10 (count + 1) worms recovered from animals offered high protein (H) or low protein (L) diets and infected with 2000 L3 *T. colubriformis* larvae d^{-1} for 77 days with an intact immunity (IF) or immuno-suppressed (ISIF). Back-transformed values are given in parenthesis

	High protein (H)		Low protein (L)		
	IF	ISIF	IF	ISIF	s.e.m
L5 Adult	3.56[a] (3,638)	4.87[b] (73,960)	4.73[b] (53,087)	4.84[b] (69,182)	0.29
L4	1.51[a] (32)	2.80[b] (633)	2.31[c] (205)	2.94[b] (866)	0.17
L3	1.72[a] (51)	3.02[b] (1,056)	2.66[b] (458)	2.68[b] (480)	0.34
Total	3.63[a] (4,242)	4.88[b] (76,469)	4.67[b] (47,254)	4.84[b] (68,488)	0.27

[a,b] Values for each stage of larval development with different superscripts are significantly different (p<0.01)

Mean lengths of male and female worms are given in Table 4.3. Overall, there was an effect of diet (p=0.003) with worms from high protein animals being shorter than worms from low protein animals. In addition, a diet x immuno-suppression interaction (p<0.001) and a diet x worm sex interaction (p=0.02) were present. The former was reflected in longer worm lengths in immuno-suppressed high protein animals but shorter lengths in their low protein counterparts. The latter was due to female worms being longer than male worms in low protein animals but not different in high protein animals, regardless of immuno-suppression treatment.

Table 4.3: Mean length of male and female worms (mm) recovered from animals offered high protein (H) or low protein (L) diets and infected with 2000 L3 *T. colubriformis* larvae d^{-1} for 77 days with an intact immunity (IF) or immuno-suppressed (ISIF)

	High protein (H)		Low protein (L)		
	IF	ISIF	IF	ISIF	s.e.m
Female	4.67[a]	5.06[b]	5.75[c]	5.32[b]	0.11
Male	4.52[a]	5.08[b]	5.2[b]	4.56[a]	
Overall	4.59[a]	5.07[b]	5.48[c]	4.94[b]	0.08

[a,b,c] Values within rows with different superscripts are significantly different ($p<0.05$)

4.3.6. Carcass composition, wool growth and nutrient utilization

Tissue weights of animals, estimated by computed tomography at day -8 and 76 of infection, and calculations of gross efficiency of energy utilization are given in Table 4.4. There were no differences between groups for any of the carcass components at day -8 ($p>0.05$). Overall, bone deposition was affected by diet ($p<0.001$) and treatment ($p=0.003$) through being reduced in low protein animals and in both IF groups, regardless of diet.

Muscle deposition was reduced in low protein animals ($p<0.001$). There was also an effect of treatment ($p=0.002$), with infection causing a 0.60 and 5.51 fold reduction in muscle deposition in HIF and LIF relative to HC and LC, respectively. Neither immuno-suppression alone nor infection in addition to immuno-suppression caused a significant reduction in muscle deposition ($p>0.05$ for both).

Fat deposition was lower in low than in high protein animals ($p<0.001$), and was also affected by treatment ($p=0.004$) with similar relative 0.33 and 0.31 reductions observed in HIF and LIF relative to HC and LC, respectively. Neither immuno-suppression nor infection in addition to immuno-suppression had any effect on fat deposition ($p>0.05$).

Clean wool production was reduced in low protein animals ($p<0.001$). Overall, there tended to be an effect of treatment ($p=0.053$) with comparative reductions of 0.12, 0.15 and 0.22 in HIF, LIF and LISIF, respectively.

Total ME intake was lower in low than in high protein animals ($p<0.001$), and was affected by treatment ($p=0.043$) through a 0.13 proportionate reduction in both HIF and LIF relative to HC and LC, respectively, but was not affected by immuno-suppression.

Total NE deposited in the carcass and wool was reduced in low compared to high protein animals ($p<0.001$). There was also an effect of treatment ($p=0.004$) with relative reductions of 0.32, 0.39, 0.21 and 0.31 observed in HIF, LIF, HISIF and LISIF, respectively, relative to their respective controls.

Overall, there was an effect of diet ($p=0.017$) and treatment ($p=0.021$) on the gross efficiency of deposition of NE from ME, reflected in reduced efficiency in low protein animals and also in IF animals that was primarily as a result of a 0.51 proportionate reduction in LIF relative to LC ($p=0.01$). No other significant reductions were observed.

Table 4.4: Computer tomographically estimated carcass growth, wool production and energy utilization in lambs offered high (H) or low (L) protein diets and infected with 2000 L3 *T. colubriformis* larvae d^{-1} (IF), similarly infected and immuno-suppressed (ISIF), immuno-suppressed only (IS) or control (C)

	High Protein (H)				Low Protein (L)				
	C	IS	ISIF	IF	C	IS	ISIF	IF	s.e.m
Original composition (day -8)									
Liveweight (kg)	27.1	27.2	28.4	27.6	27.3	27.7	27.9	27.7	0.78
Carcass weight (kg)	12.9	12.9	13.5	13.1	13.0	13.2	13.3	13.2	0.40
Bone weight (kg)	1.62	1.62	1.68	1.64	1.63	1.65	1.66	1.65	0.04
Muscle weight (kg)	9.46	9.56	9.91	9.56	9.63	9.85	9.61	9.61	0.35
Fat weight (kg)	1.78	1.70	1.89	1.88	1.67	1.63	1.99	1.91	0.22
Tissue deposition (day 76)									
Fasted liveweight gain (g d^{-1})	246^{a}	233^{ac}	151^{bcd}	138^{b}	86^{de}	104^{bde}	35^{fe}	-21^{f}	32.8
Bone deposition (kg)	0.83^{a}	0.79^{a}	0.51^{b}	0.47^{b}	0.30^{b}	0.37^{b}	0.10^{c}	-0.09^{c}	0.12
Muscle deposition (kg)	3.59^{a}	2.60^{ab}	0.83^{bc}	1.43^{bc}	0.53^{c}	0.19^{ce}	-1.44^{de}	-2.39^{d}	0.77
Fat deposition (kg)	5.40^{a}	6.01^{a}	4.77^{ac}	3.63^{bc}	2.64^{bd}	3.69^{bc}	2.7^{be}	1.81^{de}	0.52
Clean wool wt (kg)	1.42^{a}	1.31^{ab}	1.23^{b}	1.25^{ab}	0.84^{cd}	0.91^{c}	0.71^{d}	0.71^{d}	0.07
Total ME intake (MJ)	1503^{a}	1530^{a}	1411^{ab}	1305^{b}	844^{cd}	1016^{c}	885^{cd}	736^{d}	82.4
Total NE deposited (MJ)	271^{ab}	287^{a}	226^{bc}	184^{cd}	130^{de}	172^{cdf}	119^{ef}	79^{e}	25.1
NE:ME	0.18^{a}	0.19^{ab}	0.16^{ab}	0.14^{ab}	0.15^{ab}	0.17^{ab}	0.13^{b}	0.07^{c}	0.02

^{a,b,c,d,e,f} Values within rows with different superscripts are significantly different ($p<0.05$)

4.3.7. Serum total protein

Mean serum total protein concentrations are given in Figure 4.6. Overall, there was an effect of diet ($p<0.001$) reflected in lower concentrations in low protein animals. There was also a treatment x time interaction ($p<0.001$) reflected in a temporary increase in immuno-suppressed animals from day 21 to 49.

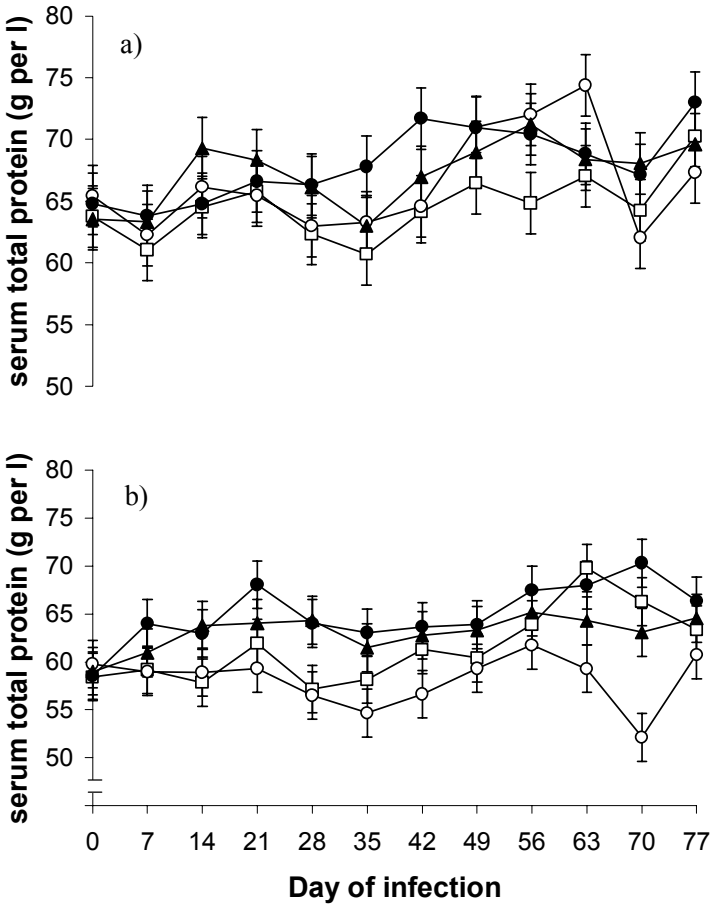

Figure 4.6: Mean serum total protein concentrations of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.3.8. Serum albumin

Mean serum albumin concentrations are given in Figure 4.7. Overall, there were diet x treatment ($p<0.001$) and treatment x time interactions ($p<0.001$). The former was reflected in a reduction in serum albumin in IF animals that was greater in those on the low protein diet. The latter was a consequence of an increase in serum albumin in all groups with time except in IF animals. Relative to their respective controls, infection reduced serum albumin concentrations in HIF by 0.11-0.32 from day 42 and in LIF by 0.15-0.37 from day 21. Immuno-suppression alone tended to increase serum albumin concentrations in high protein, but not low protein animals. Infection in addition to immuno-suppression had no effect in high protein but did result in a 0.11-0.23 relative depression in low protein animals from day 57 ($p<0.05$).

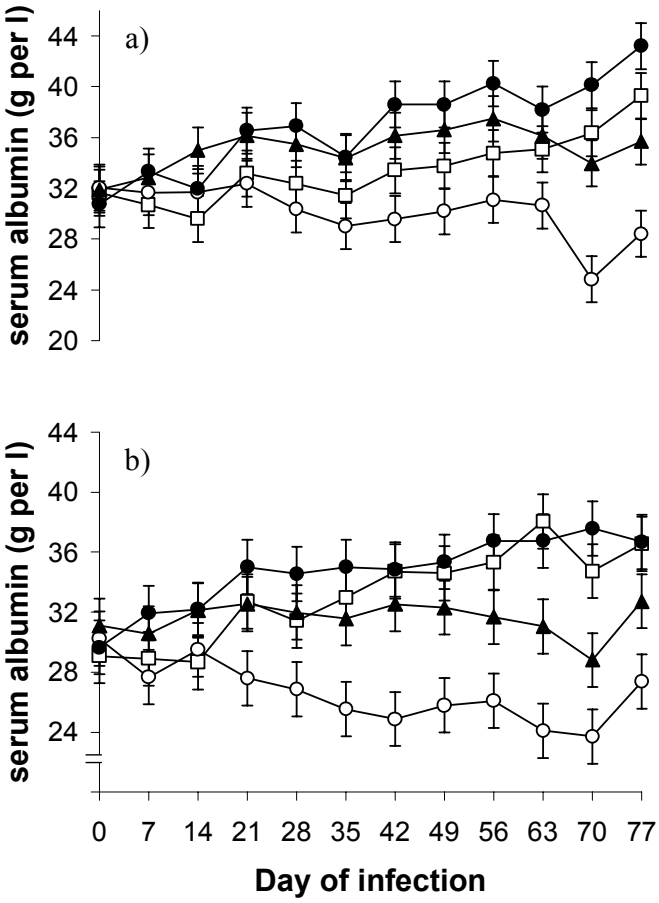

Figure 4.7: Mean serum albumin concentrations of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.3.9. Serum globulin

Mean serum globulin concentrations are given in Figure 4.8. Overall, there was a treatment x time interaction ($p<0.001$) reflected in a 0.14-0.37 relative increase in HIF and LIF from day 35 with the exception of day 70. Regardless of diet, immuno-suppression alone had no effect while infection in addition to immuno-suppression resulted in a temporary 0.22 proportionate increase on day 70 in HISIF ($p=0.04$), but not LISIF animals.

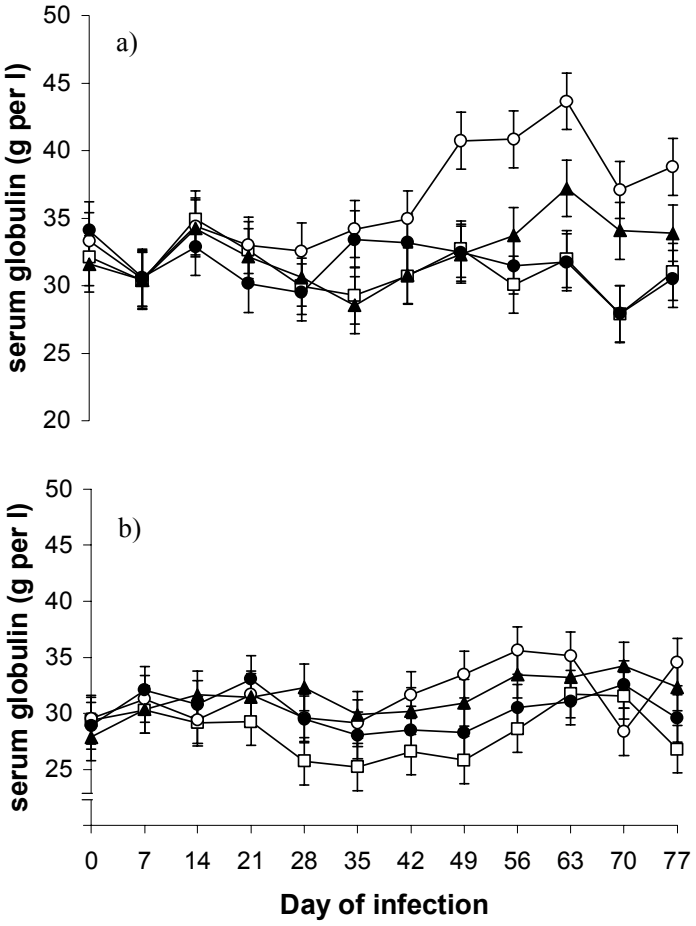

Figure 4.8: Mean serum globulin concentrations of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.3.10. Serum urea

Mean serum urea concentrations are given in Figure 4.9. There was an effect of treatment (p=0.002) with serum urea concentrations increased in IS animals only. In addition, there was a diet x time interaction (p<0.01) as a result of animals on the high protein diet having serum urea values proportionately 0.30-0.66 greater than their low protein counterparts (p<0.001) until an increase in low protein animals at day 77 that did not occur in high protein animals.

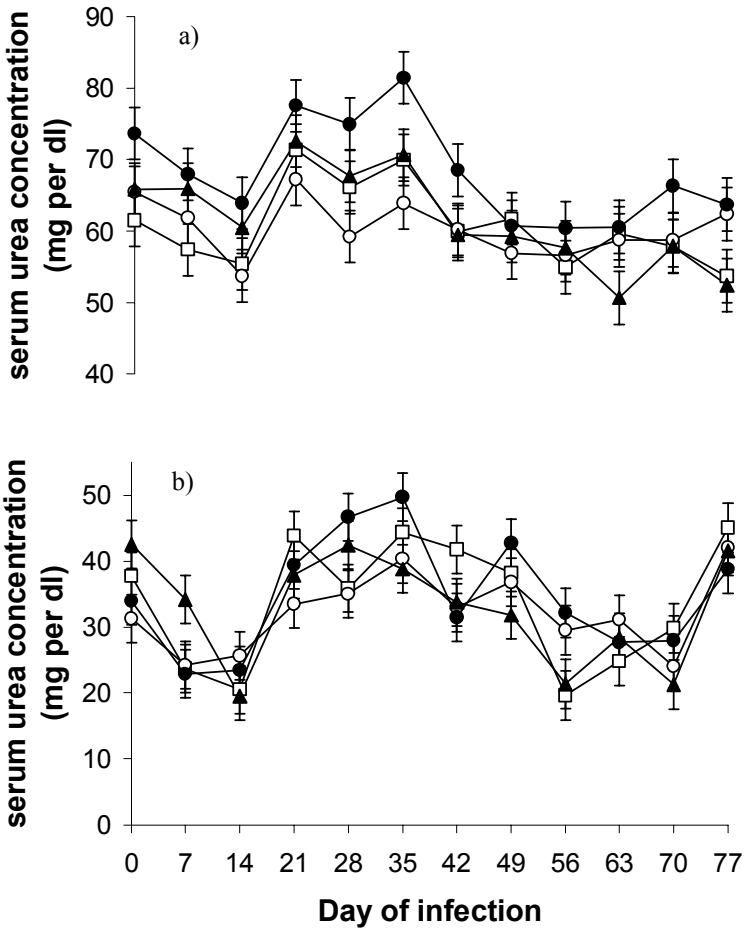

Figure 4.9: Mean serum urea concentrations of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.3.11. Serum phosphate

Mean serum phosphate concentrations are given in Figure 4.10 and were not affected by diet (p=0.194). Overall, there was a diet x time (p=0.032) and treatment x time (p<0.01) interaction that reflected a relative decrease of up to 0.50 in both IF and ISIF, but not in IS or C animals, from day 21 in low protein and day 28 in high protein fed animals, and a subsequent return to levels similar to C animals from day 70 in HIF animals only. Immuno-suppression alone had no effect in high protein animals, but did tend to reduce serum phosphate in low protein animals p=0.16).

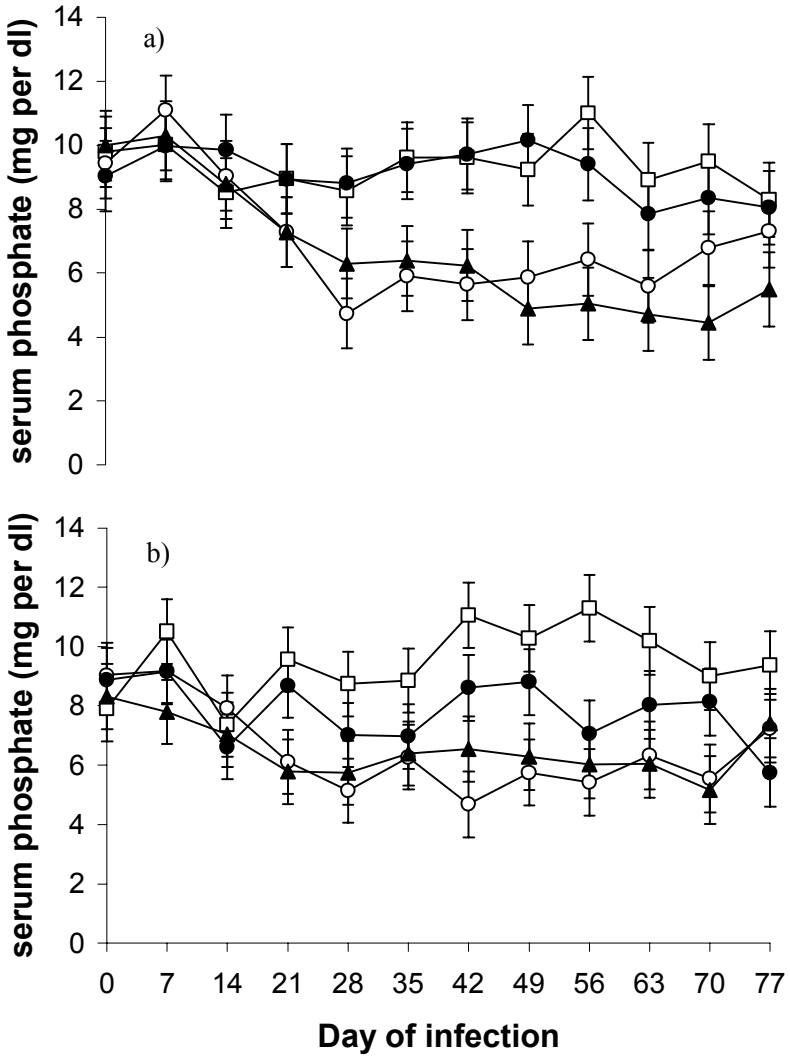

Figure 4.10: Mean serum phosphate concentrations of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.3.12. Serum total antibody

Mean absorbance for *T. colubriformis*-specific L3 serum total antibody is given in Figure 4.11. There was a diet x treatment x time interaction ($p<0.001$) as a result of a rise in IF animals from day 49 and in ISIF animals on day 77 that was greater in high protein animals than their low protein counterparts and also greater in IF compared with their ISIF counterparts. Immuno-suppression alone had no effect on total antibody regardless of diet as IS remained at all time similar to C animals.

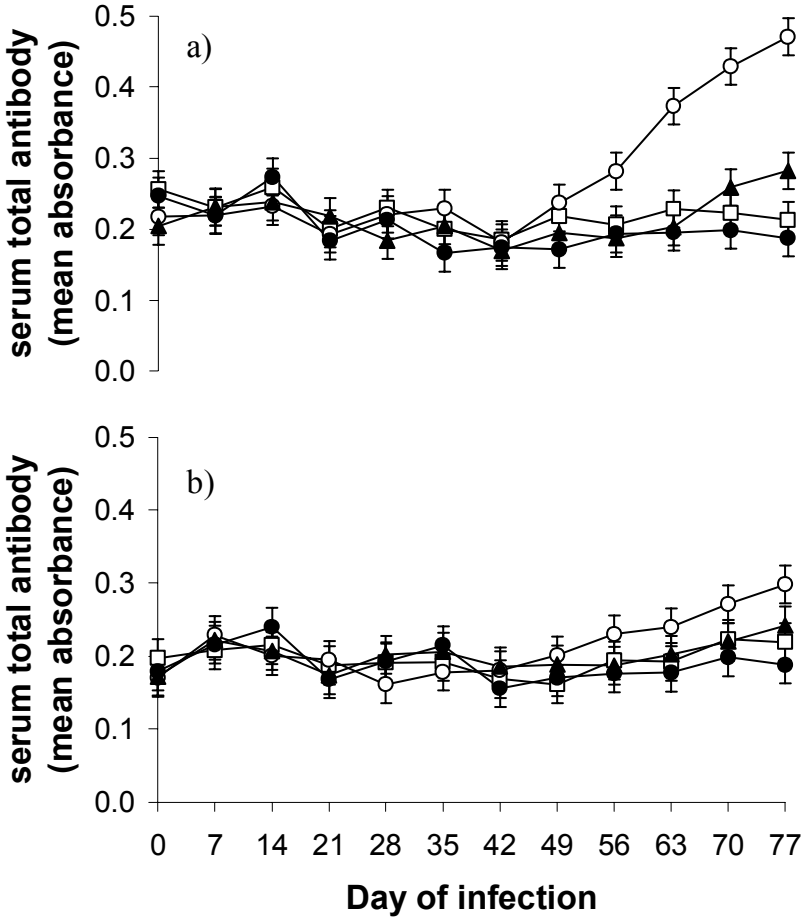

Figure 4.11: Mean absorbance of serum total L3 antibody of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.3.13. Serum immunoglobulin A

Mean serum *T. colubriformis*-specific L3 IgA absorbance is given in Figure 4.12. Overall, there was an effect of treatment ($p<0.001$), time ($p<0.001$) and a treatment x time interaction ($p<0.001$) due to a rise in IgA in IF and ISIF animals from day 49 that was comparable between diets but greater in ISIF than in IF animals.

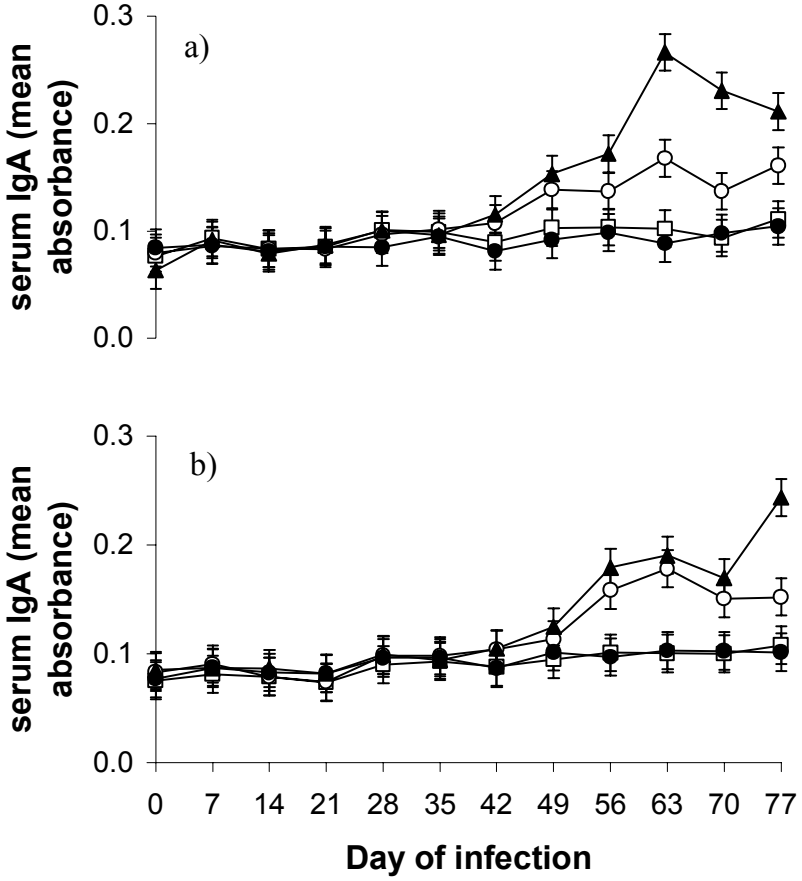

Figure 4.12: Mean absorbance of serum L3 IgA of lambs offered a) high protein or b) low protein diets while ○ infected with 2000 *T. colubriformis* d^{-1} (IF) , ▲ similarly infected but immuno-suppressed (ISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

4.4. Discussion

These findings support the observations in Chapter 3 that physiological changes accompanying the acquisition phase of the immune response are implicated in the reduction in feed intake and nutrient utilization in parasitised lambs. Moreover, the findings suggest that the extent of metabolic disruption caused as a consequence of the physiological changes associated with the development of immunity is moderated through the provision of adequate protein.

The current infection regime can be considered to have been effective in providing a pathogenic infection. While the lower worm burdens (Table 4.2) in HIF than in LIF animals at slaughter may be expected due to the development of immunity in these animals that was reflected in a decrease in total egg output from day 56 (Figure 4.5), mean worm burdens of 47,000 in LIF animals are comparable to the 45-51,000 found by Sykes and Coop (1976) and by Bown *et al.* (1991a) using similar rates of infection with this nematode in naïve lambs. In addition, the worm burdens of 76,500 and 68,000 in HISIF and LISIF are greater than the 22,000 to 25,000 observed in animals receiving a similar immuno-suppression regime in Chapter 3, but are still much lower than the 267,000 found by Nagasinha (1999). Nevertheless, the total nematode output from IF animals of 500,000 to 700,000 eggs d^{-1} and the greater than 1,000,000 eggs d^{-1} from ISIF animals suggest a substantial infection was established. Support for this interpretation can be observed in the 0.50 proportionate reduction in serum phosphate in all animals which coincided with the presence of an adult parasite burden from around three weeks of infection (Figure 4.10), a reduction that was comparable, both in magnitude and temporally to the observations of Coop *et al*. (1976), Sykes *et al*. (1979) and Bown *et al*. (1989). Hypophosphataemia is typical of small intestinal parasite infections and is considered a consequence of intestinal pathology that results in an impairment of phosphorous absorption (Coop and Field, 1983; Wilson and Field, 1983, Bown *et al*., 1989). Furthermore, pathology of the intestine can result in greater leakage of plasma proteins into

the alimentary tract that is reflected in reduced serum albumin concentrations that can not be explained through displacement with serum globulin (Steel *et al*., 1980; Jones and Symons, 1982; Kimambo *et al*., 1988; Bown *et al*., 1991b). The reduction of serum albumin in LIF animals (Figure 4.7) was again comparable in magnitude and temporally with the observations of previous workers (Steel *et al*., 1980; Bown *et al*., 1991b), and could not be explained by an increase of serum globulin until day 42, suggesting the hypoalbumaenia in LIF during the first six weeks of infection was due to increased leakage as a consequence of intestinal damage. The absence of a reduction in serum albumin in HIF or both immuno-suppressed and infected groups is likely to be due to an improved ability to replace leaked proteins through a greater supply of available protein, rather than a lessening of protein leakage (Jones and Symons, 1982). Infection also caused a reduction in fasted liveweight gain of 108 and 107g d^{-1} in HIF and LIF, respectively, indicating the pathogenicity of infection was not just reflected in changes in nutrient absorption/leakage in the gut, but also in animal performance.

Provision of additional MP enhanced the development of an effective immunological response. Infected animals on the low protein diet (LIF) did not appear to have developed an effective immunity, displaying no apparent reduction in total faecal egg excretion and having worm burdens at slaughter comparable with immuno-suppressed infected animals. In comparison, additional protein in HIF animals allowed the establishment of a competent immune response, as shown by the typical decline in faecal nematode egg excretion from day 56 and the considerably lower worm burden at slaughter. Protein supply undoubtedly affects the ability of the host to deliver an effective immune response (Sykes and Coop, 2001), while the comparable worm burdens in HISIF and LISIF sheep, combined with the similar total nematode egg outputs of HIF and LIF until day 56 (Figure 4.5) provide strong evidence that parasite establishment or viability was not influenced through a direct anthelmintic effect of the high protein diet. van Houtert *et al*. (1995) also

reported that larval establishment rates were not affected with supplementation of up to 100g fishmeal d^{-1}. Furthermore, post-ruminal infusions of casein by Bown *et al.* (1991a) which supplied an additional 50g d^{-1} of MP to a basal diet of approximately 50g d^{-1} MP while not changing innate immunity, that was reflected in a lack of difference in worm burdens after six weeks of infection, did allow the subsequent development of a protective immune response after 12 weeks of infection with *T. colubriformis*. The ability of HIF animals in the current study to promote an effective immune response may, therefore, be expected as these animals, on average, received 173g d^{-1} MP, which was considerably greater than the 63g d^{-1} consumed by their LIF counterparts. The apparent increase in immune responsiveness of HIF compared with LIF animals was reflected in a larger total antibody response from day 56 that corresponded with a decrease in total egg production, but additional protein did not increase L3 IgA production, indicating L3 IgA was not sensitive to changes in protein supply. This is in contrast to the findings of Strain and Stear (2001) who observed in animals infected with *Haemonchus contortus* that compared to their counterparts consuming a diet supplying 98gMP $kgDM^{-1}$, those offered a diet supplying 173gMP $kgDM^{-1}$ exhibited a 2 fold greater L3 IgA response while having comparable worm burdens. In addition, these authors observed a reduction in mean worm length from 2.13 to 2.00cm in the high protein animals that was attributed almost entirely to the difference in parasite-specific IgA. While reductions in worm length in the current study (Table 4.3) were apparent in HIF compared with both LIF and HISIF, female worm length did not appear to be influenced by peak serum L3 IgA (R^2=0.11). In contrast, Smith *et al*. (1983) reported a strong negative correlation (R^2=0.92) between peak lymph IgA at day 6 of infection with *O. circumcincta* and worm length, which has been found by Stear and Bishop (1999) to be associated with worm fecundity. Calculation of average daily egg excretion per female at the time of slaughter in the current study indicates egg production was 2 and 24 eggs d^{-1} for HIF and LIF, respectively, suggesting that the immune response suppressed egg production in HIF animals independent of L3 IgA concentrations in plasma.

While the discrepancy between the results reported here and those in the literature may be due to the alternative method of calculating female fecundity, as the previously mentioned authors measured eggs *in utero* of female worms at slaughter and would not have an indication of the rate of egg production, it does appear that the reduced female fecundity, length and burden of worms in HIF compared with LIF can be attributed to components of the immune response in the effecter mechanism other than IgA that were enhanced in a high protein environment.

The provision of additional MP appeared to reduce the metabolic disturbance incurred as a consequence of infection. The proportional reduction in the gross efficiency of metabolizable energy (ME) utilization of 0.50 in LIF animals (Table 4.4) consuming a diet containing 93g crude protein (CP) $kgDM^{-1}$ is almost identical to that found by Sykes and Coop (1976) in growing lambs receiving similar rates of infection with this nematode while consuming a diet containing 140gCP $kgDM^{-1}$. In addition, the reduction in ME utilization of 0.23 in HIF animals consuming a diet containing 175gCP $kgDM^{-1}$ is comparable to the 0.20 reduction observed in Chapter 3 in lambs consuming a diet containing 205gCP $kgDM^{-1}$. Despite similar 0.13 proportionate reductions in total ME intake, the lesser reduction of ME utilization in HIF compared with LIF animals implies that increases in protein supply enabled the processes which enhanced the efficiency of nutrient utilization, possibly reflective of the rapid development of a less metabolically disruptive Th-2 type immune reaction. The energetic cost of infection reflected in the proportional reduction in adipose tissue deposition of 0.33 and 0.31 in HIF and LIF, respectively, did not appear to be influenced by protein supply. The reductions in the rate of adipose deposition are comparable with the 0.38 reduction observed by Sykes and Coop (1976) and that of 0.37 reported in Chapter 3. However, the deamination of amino acids as a consequence of net loss of muscle tissue in LIF animals would be expected to have provided an additional 57 MJME (AFRC, 1993), suggesting that the energetic cost of infection was greater in LIF animals. The greater muscle

deposition in HIF animals is not surprising, and presumably reflects an enhanced dietary supply of potentially rate-limiting amino acids that are required for the replacement of leaked plasma proteins into the alimentary tract or the production of protein rich immunoglobulins, mucoproteins and leukotrienes involved in the effecter arm of the immune response (Coop and Holmes, 1996). Reductions in wool growth of 0.11 have been observed in animals that are resistant to infection with *T. colubriformis* (Barger and Southcott, 1975) which has been attributed to competition between the immune system and wool production for sulphur amino acids (MacRae, 1993). It is surprising, therefore, that HIF and LIF had comparable depressions in wool production of 0.12 and 0.15, respectively, as a more pronounced reduction in wool growth may have been anticipated in protein restricted LIF animals. Despite an apparent lack of ability to promote an effective immunological reaction, it is interesting that LIF animals still exhibited a greater metabolic cost of infection than their HIF counterparts that was reflected in a larger reduction in both liveweight gain and gross efficiency of ME utilization. This possibly suggests that the nutritional cost of infection may be attributed to the demands of immunological mechanisms other than those involved directly with the expulsion of nematodes and which are less metabolically disruptive in animals offered a high protein diet. This will be discussed in more detail later.

Corticosteroid treatment appeared to reduce the metabolic disturbance of infection. Despite similar levels of hypophosphataemia in all infected groups, indicating comparable intestinal pathology, animals that were concurrently immuno-suppressed and infected suffered no reduction in liveweight gain or efficiency of ME utilization other than can be explained by the effect of the corticosteroid alone in IS animals. Reductions in liveweight gain as a consequence of corticosteroid treatment alone that were observed in the first trial of this series (Chapter 3) were not evident in either HIS or LIS animals. Nevertheless, both HIS and LIS did experience comparable reductions in muscle deposition and increases in fat deposition observed in immuno-

suppressed animals in Chapter 3. The former is probably a consequence of the net catabolic effect of corticosteroids on muscle tissue (Huang *et al*., 1998) and wool production (Paranetto, 1979), while the latter (increases in fat deposition) has been observed previously in dexamethasone treated cattle (Dicke *et al*. 1974; Corah *et al*. 1995) and presumably reflects an increased energy supply from deaminated carbon skeletons coupled with a reduced ME demand for protein synthesis. Turini *et al*. (2003) found fractional protein synthesis rates were reduced in the muscle and intestine and increased in the liver of dexamethasone treated rats, resulting in larger livers. Evidence for this in the current study can be observed in a greater serum urea concentrations in IS animals and in the lack of a reduction in serum albumin in ISIF animals in addition to larger livers at slaughter of immuno-suppressed animals. The requirement for protein for the replacement of leaked albumin as a consequence of intestinal pathology may explain the reduced wool production observed in LISIF animals. Support for this can be observed in the net muscle loss and the slight reduction in serum albumin in LISIF sheep, suggesting these animals were not quite able to match the loss of serum proteins into the alimentary tract. Nevertheless, despite harbouring much larger worm burdens at slaughter, infection in addition to immuno-suppression did not result in significant reductions in muscle or adipose deposition, suggesting very little energy was invested into the repair of damage to the alimentary tract. This cannot be explained through an increase in ME from the diet as there was no effect of immuno-suppression on DM digestibility, nor is infection with this nematode likely to affect DM digestibility (Sykes and Coop, 1976; Bown *et al*., 1991b). These results indicate infection in immuno-suppressed animals did not cause a major metabolic disturbance other than that which can be explained by the actions of the corticosteroid compounds alone.

Animals offered the high protein diet suffered a lesser reduction in feed intake than their low protein counterparts. The reduction in feed intake during nematode parasite infections has been implicated in 0.60-0.90 of the production losses observed in young lambs (van Houtert and Sykes, 1996). Consequently, the cause and reasons for this aspect have received considerable attention (Dynes *et al*. 1998; Kyriazakis *et al*., 1998). In spite of this, the cause of reduced appetite in infected lambs has been elusive, but appears to be associated with the presence of adult nematodes in the gut as reduced feed intake in studies with this nematode has been shown to occur from three weeks of infection, which is the time taken for larvae to develop to adults, and to return to normal after anthelmintic treatment or the development of an effective immune response (Kyriazakis *et al*., 1996, Kimambo *et al*., 1988). The average depression of appetite from days 22 to 63 of infection in the current trial of 0.12 and 0.23 for HIF and LIF, respectively (Figure 4.2) is comparable to what may be expected with this level of infection of this nematode (Sykes and Coop, 1976; Steel *et al*., 1980). Feed intake of LIF would not have been expected to improve by the conclusion of the trial due to the apparent inability of these animals to promote an effective immune response. However, it is surprising that intakes of HIF animals did not recover as the worm burdens and nematode egg excretion of these animals suggest they were capable of expelling the nematode. The lesser proportionate reduction in feed intake of animals offered high protein diets have been observed in the past (Kyriazakis *et al*., 1996) and are thought to be due to reduced damage of the gastro-intestinal tract as a consequence of an enhanced immunological response (van Houtert and Sykes, 1996). It is interesting that HIF animals experienced a lesser reduction in intake than their low protein counterparts, as comparable total daily egg production to LIF may be expected to indicate the presence of similar adult worm numbers until an effective immunological response was developed, indicated by the reduction in total egg output from day 56. These results indicate that the additional protein supplied to HIF animals influenced the reduction of intake through mechanisms other than a reduced parasite burden.

Immuno-suppression alleviated the reduction of intake associated with nematode infection. Corticosteroids appear to have varied effects on the intake in sheep, with small single doses of 0.1mg dexamethasone kg^{-1} (equivalent to 0.5mg methylprednisolone) stimulating short term increases (Adams and Sanders, 1992) while larger single doses of 6mg dexamethasone kg^{-1} have resulted in a short term depression of intake (Paranetto, 1979). The chronic weekly administration of 1.3mg $kgLW^{-1}$ in the current study had no significant effect on intake in IS animals, although there was a tendency for it to be reduced in HIS animals. Notably, infection in addition to immuno-suppression caused no detrimental effect on intake, with ISIF animals from both protein groups consuming food at rates that were not significantly different to their IS counterparts throughout. This lack of a reduction in intake in ISIF animals may be surprising, as these animals were harbouring much larger worm burdens at slaughter than their immunologically normal counterparts. The result of the current study is in agreement with previous observations in Chapter 3, in which corticosteroid induced immuno-suppression prevented a 0.30 decrease in voluntary feed intake during infection with this nematode. Although the exact mechanism cannot be elucidated at this stage, these results provide further evidence that the reduction of appetite may be as a consequence of the physiological signalling associated with the developing immune response, rather than the physical damage caused to the gastro-intestinal tract by the parasite *per se*. While there may be little supporting evidence from sheep, human and murine studies have identified pro-inflammatory cytokines involved in the acute phase response (APR) such as interferon (IFN)-γ, interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α that cause anorexia in exposed subjects (Johnson, 1998; Langhans, 2000; Farthing and Ballinger, 2001). In addition, Ing *et al.* (2000) found protein malnourished mice infected with the nematode *Heligmosomoides polygyrus* exhibited a cytokine response characteristic of a pro-inflammatory Th-1 type reaction, while those on a protein sufficient diet displayed a cytokine response indicative of a Th-2 type cascade that resulted in reduced worm burdens. This may provide a functional

explanation for the greater reduction of feed intake in LIF animals compared with HIF as the lack of available protein rendered them capable of mounting primarily only a pro-inflammatory Th-1 type immunological reaction, whereas the greater supply of protein in HIF animals may have furthered the development of an effective Th-2 type reaction, consequently these animals were less dependant on the APR and the consequences of its cytokine cascade. Although there still may be some question about the exact cytokine cascade, and the role of each component during the ovine immunological response to nematode parasites, the current trial provides further evidence that constituents of the developing immune response are a causative factor in the reduction in feed intake during infection with this nematode parasite, the exact degree of which appears to be influenced by protein supply.

The regime of weekly intramuscular injections of 1.3mg methylprednisolone acetate $kgLW^{-1}$ has previously provided effective immuno-suppression (Nagasinha, 1999). However, for reasons that are not apparent, during the current trial a number of the animals in both the high and low protein ISIF groups were suspected not to be responding to the immuno-suppressive treatment. This was characterised by a reduced intake in some ISIF individuals most notably in the days prior to weekly corticosteroid treatment that would then increase for several days following treatment. The pattern of intake for one animal (No 20) is shown in Figure 4.13. Despite corticosteroid treatment allowing the development of large worm burdens and suppressing the production of total L3 antibody, analysis of serum L3 IgA suggested incomplete immuno-suppression with seven HISIF and four LISIF promoting an IgA response. On the other hand, four of the eight IF animals from each of the L and H diets did not express an IgA profile, the reasons for which are not evident, as average worm burdens of 2,350 in the four high protein individuals in question suggest they had mounted a competent immune response. Consequently, infected animals within each dietary treatment, regardless of corticosteroid treatment, were termed IgA responders (R) if they promoted an

IgA response that was greater than three standard deviations from the mean of their respective controls (C or IS animals) for two consecutive blood samples. Infected individuals that did not promote such an IgA response were termed as non-responders (N). This resulted in the 16 H and 16 L infected animals being divided into high protein IgA non-responder (HN; n=5), high protein IgA responder (HR; n=11), low protein IgA non-responder (LN; n=8) and low protein IgA responder (LR; n=8), with the uninfected animals for each dietary treatment combined into high protein uninfected (HC+IS; n=10) and low protein uninfected (LC+IS; n=10), the serum L3 IgA profiles from which are shown in Figure 4.14.

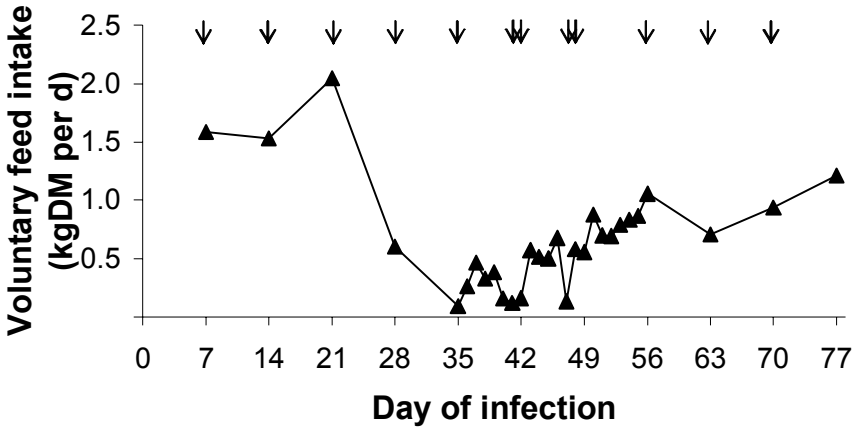

Figure 4.13: Daily voluntary feed intake of a LISIF individual (No 20) displaying short-term effects of immuno-suppressive treatment. Daily feed intake was monitored from day 35 to 56. Arrows indicate weekly steroid administration, with additional doses given on days 41 and 48.

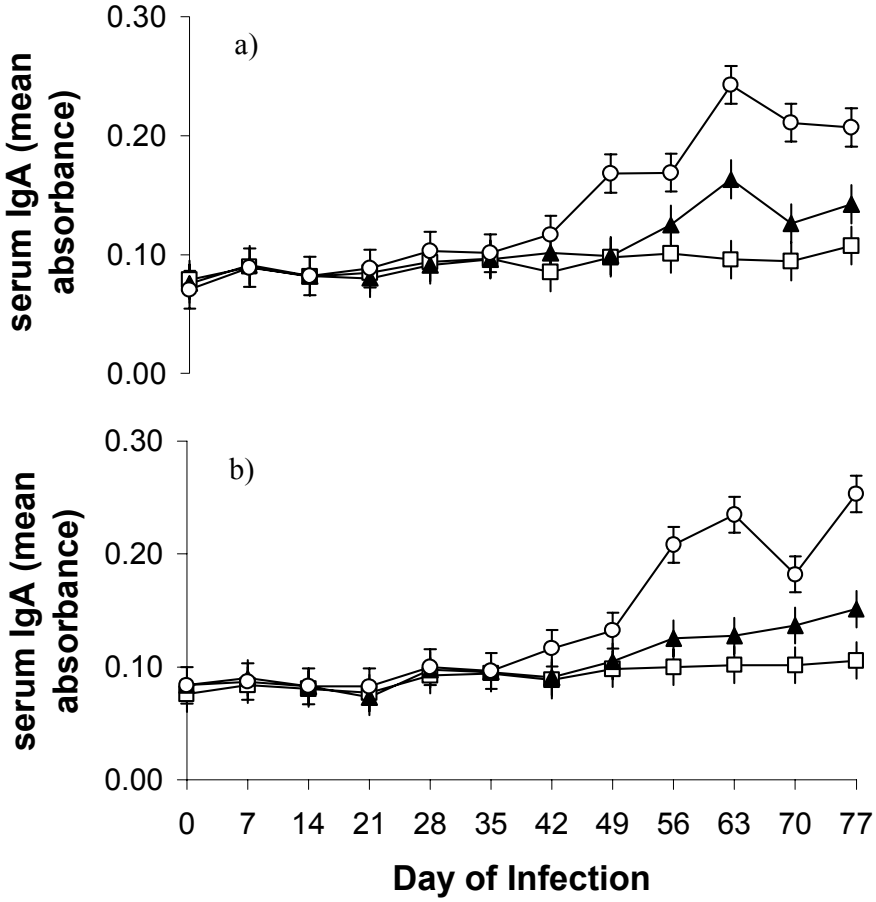

Figure 4.14: Mean absorbance of serum L3 IgA of lambs offered a) high protein or b) low protein diets that ○ displayed an L3 IgA response after infection with 2000 *T. colubriformis* d^{-1} (R) , ▲ were similarly infected but did not display an L3 IgA response (N) or □ immuno-suppressed only and controls combined (IS+C).

Division of animals into IgA responders and non-responders allows the suggestion that L3 IgA production may be associated with the metabolic cost of infection, particularly in animals on a low protein diet. Reductions in feed intake from day 22 averaged 0.15 for HR and 0.32 for LR groups compared to HC+IS and LC+IS, respectively (Figure 4.15). In comparison, HN averaged a 0.08 reduction while LN suffered no reduction at all. Despite the 0.21 reduction in liveweight gain (Figure 4.16) resulting in a 0.09 lighter fasted liveweight of HR compared with HN at day 78 ($p=0.05$), this was not reflected in differences in carcass tissue deposition or gross utilization of ME (Table 4.5). In contrast, LR animals suffered a greater metabolic disturbance with significant reductions

in liveweight gain and net energy deposition that culminated in a 0.59 reduction in gross utilization of ME, neither of which was observed in LN animals. This decrease in ME utilization in LR animals was a consequence of significant 8-fold reductions in muscle, 0.48 reductions in fat and 0.26 reduced wool deposition, all of which were not apparent in LN, HR or HN groups. These results are comparable to those described in Chapter 3, as both immuno-suppressed 5-month-old lambs and 17-month-old immunologically competent ewes did not display an IgA profile or suffer a reduction in intake or performance after infection with *T. colubriformis*. In addition, a reduction in efficiency of ME utilization and feed intake was observed in immunologically normal 5-month-old lambs and an aberrant individual that did not appear to be responsive to immuno-suppressive treatment, both of which displayed an L3 IgA response. Zhou *et al*. (2005) have identified three variants of the ovine IgA allele, with the IgA molecule of all three having a hinge region that is comparatively cysteine rich. Production of IgA in large enough quantities may result in a considerable demand for rate limiting amino acids. The competition for potentially rate-limiting amino acids between the immune response and productive processes in the body may provide a functional explanation for the greater reduction in muscle deposition and cysteine-rich wool production observed in LR animals. In addition, the pro-inflammatory cytokine IL-6 is known to play a key role in promoting IgA proliferation (Ramsay *et al*., 1994; Husband *et al*., 1996) and has been found to have no correlation with serum values and feed intake, but strong negative correlations for both weight gain (-0.832) and protein accretion (-0.720) in pigs seven days after inoculation with Porcine Reproductive and Respiratory Syndrome Virus (Escobar *et al*., 2004). Although further elucidation is required, it is apparent that components of the immune response associated with L3 IgA appear to contribute to the metabolic cost of infection, particularly in animals with a limited protein supply. Furthermore, these results suggest that sufficient protein supply may alleviate the nutritional disturbance linked with this component of the immune response.

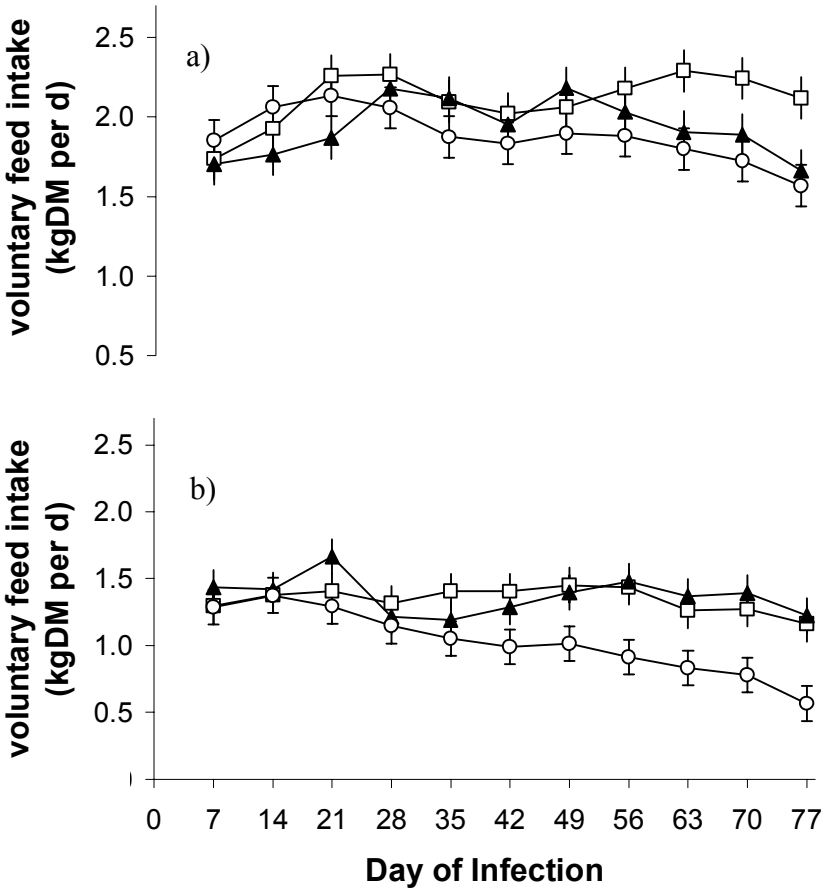

Figure 4.15: Mean daily voluntary feed intakes of lambs offered a) high protein or b) low protein diets that ○ displayed an L3 IgA response after infection with 2000 *T. colubriformis* d^{-1} (R) , ▲ were similarly infected but did not display an L3 IgA response (N) or □ immuno-suppressed only and controls combined (IS+C).

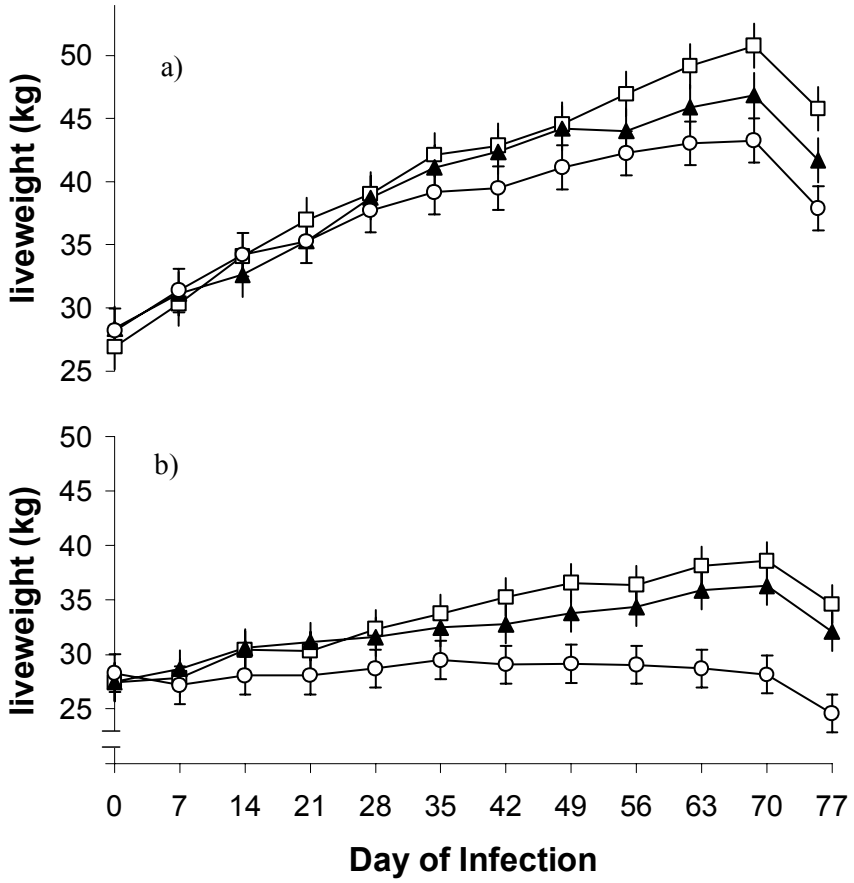

Figure 4.16: Mean liveweight of lambs offered a) high protein or b) low protein diets that ○ displayed an L3 IgA response after infection with 2000 *T. colubriformis* d^{-1} (R) , ▲ were similarly infected but did not display an L3 IgA response (N) or □ immuno-suppressed only and controls combined (IS+C).

Table 4.5: Computer tomographically estimated carcass growth, wool production and energy utilization of lambs offered high (H) or low (L) protein diets that responded with an L3 IgA response to an infection with 2000 L3 *T. colubriformis* larvae d^{-1} (R), similarly infected but did not respond (N) or immuno-suppressed and control animals that were not infected (IS+C)

	High Protein (H)			Low Protein (L)			
	IS+C	N	R	IS+C	N	R	s.e.m
Original composition (day -8)							
Liveweight (kg)	27.1	28.4	27.8	27.5	27.5	28.1	0.78
Carcass weight (kg)	12.9	13.6	13.2	13.1	13.1	13.4	0.40
Bone weight (kg)	1.62	1.68	1.65	1.64	1.64	1.67	0.04
Muscle weight (kg)	9.50	10.1	9.57	9.72	9.42	9.79	0.35
Fat weight (kg)	1.75	1.68	1.98	1.65	2.01	1.90	0.22
Tissue deposition (day 76)							
Fasted liveweight gain (g d^{-1})	243^{a}	167^{b}	133^{bc}	92^{cd}	65^{d}	-40^{e}	24.9
Bone deposition (kg)	0.82^{a}	0.57^{b}	0.45^{bc}	0.32^{cd}	0.23^{d}	-0.17^{e}	0.09
Muscle deposition (kg)	3.30^{a}	1.77^{ab}	0.80^{b}	0.42^{bc}	-0.67^{c}	-2.96^{d}	0.59
Fat deposition (kg)	5.62^{a}	4.39^{b}	4.09^{bc}	2.98^{d}	3.12^{cd}	1.56^{e}	0.44
Clean wool wt (kg)	1.39^{a}	1.31^{ab}	1.20^{b}	0.86^{c}	0.79^{c}	0.64^{d}	0.06
Total ME intake (MJ)	1516^{a}	1377^{ab}	1347^{b}	900^{c}	959^{c}	693^{d}	65.8
Total NE deposited (MJ)	277^{a}	218^{b}	198^{b}	143^{c}	142^{c}	65^{d}	20.2
NE:ME	0.18^{a}	0.16^{a}	0.15^{a}	0.16^{a}	0.15^{a}	0.06^{b}	0.02

^{a,b,c,d,e} Values within rows with different superscripts are significantly different ($p<0.05$)

Table 4.6*:* Numbers of log10 (count + 1) worms recovered from animals offered high protein (H) or low protein (L) diets and infected with 2000 L3 *T. colubriformis* larvae d^{-1} for 77 days that displayed an L3 IgA response (R) or were similarly infected but did not display an IgA response (N). Back-transformed means are shown in parenthesis

	High protein (H)		Low protein (L)		
	R	N	R	N	s.e.m
n	11	5	8	8	
L5 Adult	4.46^{ab} (28,313)	3.72^{b} (5,247)	4.79^{a} (61,943)	4.77^{a} (59,019)	0.51
L4	2.33^{ab} (215)	1.8^{b} (62)	2.72^{a} (524)	2.6^{a} (394)	0.39
L3	2.57^{a} (369)	1.98^{a} (94)	2.94^{a} (862)	2.38^{a} (239)	0.56
Total	4.47^{ab} (29,298)	3.76^{b} (5,717)	4.74^{a} (54,382)	4.80^{a} (62,457)	0.22

^{a,b} Values with different superscripts are significantly different ($p<0.05$)

Table 4.7*:* Mean length of male and female worms recovered from animals offered high protein (H) or low protein (L) diets and infected with 2000 L3 *T. colubriformis* larvae d^{-1} for 77 days that displayed an L3 IgA response (R) or were similarly infected but did not display an IgA response (N)

	High protein (H)		Low protein (L)		
	R	N	R	N	s.e.m
Female	4.95^{abc}	4.62^{a}	5.24^{c}	5.86^{d}	0.14
Male	4.78^{ab}	4.90^{bc}	4.76^{ab}	5.06^{ac}	

^{a,b,c,d} Values with different superscripts are significantly different ($p<0.05$)

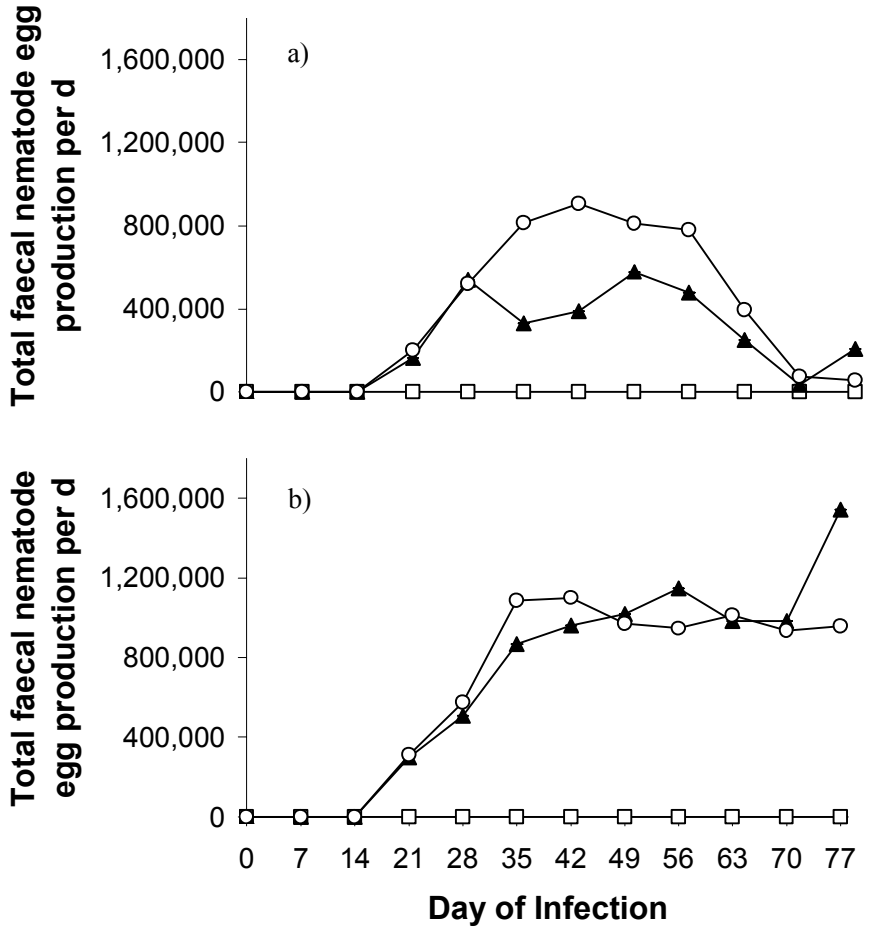

Figure 4.17: Mean back-transformed (log10 (count + 1)) total daily nematode egg production of lambs offered a) high protein or b) low protein diets that ○ displayed an L3 IgA response after infection with 2000 *T. colubriformis* d^{-1} (R) , ▲ were similarly infected but did not display an L3 IgA response (N) or □ immuno-suppressed only and controls combined (IS+C).

Immunoglobulin A is believed to have a functional role in the regulation of worm length and fecundity and expulsion of nematodes (Smith *et al*., 1983; Stear *et al*., 1999). Despite large differences in FEC, total daily nematode egg production (Figure 4.5) indicates egg output for LIF and HIF animals was similar until day 63 of infection, from which point the difference between groups was caused by decreased egg output in HIF that would be expected as a consequence of parasite expulsion, as indicated by the comparatively low worm burdens at slaughter. On the other hand, LIF animals displayed only half the total egg output of LISIF, despite having similar worm burdens at slaughter, suggesting that LIF animals were able to mount some form of protective response that was capable of suppressing egg production, but not parasite expulsion. While this may have been expected due to the rise in L3 IgA production from day 56 in LIF animals, it is interesting to note that both LISIF and HISIF animals did not appear to experience any suppression of total egg production, despite similar IgA profiles to LIF and HIF, respectively. Furthermore, regardless of diet, it would be expected that IgA responders (R) would have suppressed total egg production to a greater degree than non-responders (N). However, this is not apparent (Figure 4.17). Moreover, neither worm burden nor worm length appeared to be reduced in animals that were classified as IgA responders (Tables 4.6 and 4.7, respectively), although female worms were longer in LN animals. These results are in contrast to the previously reported associated between IgA and both worm length and fecundity in the abomasal parasites *H. contortus* and *O. circumcincta* (Smith *et al*., 1983; Stear *et al*., 1999; Strain and Stear, 2001). Zhou *et al*., (2005) suggest that differences in the IgA variants at the hinge region may result in divergence of susceptibility/resistance to parasitic or bacterial proteases. If the predominant IgA variant produced during parasitic challenge was susceptible to cleavage from parasitic proteases at the hinge region, a situation of hyper-stimulation and production of non-effective IgA can be envisaged. Such situations may provide a functional explanation for the apparent ineffective L3 IgA response in the current study that was associated with a considerable metabolic

disturbance. Alternatively, the discrepancy between these findings and other published reports from abomasal infections may reflect differences in the effectiveness of immunological components associated with responses in the abomasum and small intestine. Nevertheless, from these observations it appears that L3 IgA may not necessarily have an effective regulatory role in worm expulsion, length or fecundity of *T. colubriformis*.

In summary, despite the complex nature of the immune system and the unlikely scenario that individual components act independently, these results do provide evidence that L3 IgA does not appear to have a functional role in the development of a protective immune response or regulation of the worm population during infections with *T. colubriformis* in sheep. Furthermore, while increasing protein supply enabled further development of the effecter mechanisms of the immune response, it also alleviated the metabolic disturbance of infection as a consequence of the animals own immune reaction that was displayed through a reduction in feed intake and nutrient utilization, both of which appeared to be associated with L3 IgA. It is hypothesised that the lessening of the nutritional disturbance caused by infection observed in high protein and immuno-suppressed animals could be a consequence of altered cytokine expression during the immunological cascade that allowed a rapid progression to a Th2 response. This seems worthy of more detailed investigation.

Chapter 5

The effect of corticosteroid induced immuno-suppression on feed intake, nutrient utilization the development of immunological mechanisms during infection with the abomasal parasite *Teladorsagia circumcincta* in lambs

5.1. Introduction

The previous two studies (Chapters 3 and 4) have demonstrated that, when immune responses are suppressed, infection with the intestinal parasite *Trichostrongylus colubriformis* caused no apparent reduction in feed intake, growth rate and had little effect on nutrient utilization other than changes which could be explained by the effects of the corticosteroids alone. The small intestine is only one of the organs commonly infected with parasites, and it is not known if this effect is specific to the small intestine and/or the range of nematodes inhabiting this organ or a general effect operating in other areas of the gastro-intestinal tract and their particular nematode infections. In particular, parasites residing in the abomasum behave differently to those in the small intestine by burrowing into and damaging the tissue and glands, causing an increase in the pH of abomasal fluid (McLeay *et al.*, 1973; Anderson *et al.*, 1976; Titchen and Anderson, 1977). On the other hand, the small intestine has been considered to be much more immunologically capable than the abomasum as judged by the presence of Peyers patches and the greater numbers of globule leukocytes found in the mucosa of the small intestine compared with the abomasum in both immunized and genetically resistant sheep (Bendixsen, *et al.*, 1995; Stankiewicz *et al.*, 1995; Douch *et al.*, 1996b), although the differential cellular response may also represent the greater ability of intestinal than that of abomasal parasites to elicit antigenic stimulation.

The aim of this trial was therefore, two-fold, firstly to determine whether suppression of immunity reduces the cost of infection in the abomasum, as observed in the intestine, to establish whether the concept that the immune response is the major cost of infection is a universal phenomenon. Secondly, to enable an insight into the immunological changes at the interface between the nematode and animal tissue that may be associated with the nutritionally disruptive component(s) of the immune response by serial biopsy sampling, since abomasal biopsy sampling has been shown by Pfeffer *et al*. (1996) and by Huntley *et al*. (2004) to provide reliable and repeatable samples from fewer animals while causing a minimum amount of disruption or apparent discomfort.

5.2. Materials and methods

5.2.1. Animals and treatments

Forty, six-week-old Coopworth ewe lambs were weaned and removed from pasture to indoor pens until the start of the trial at six months of age to minimise exposure to nematode larvae. Upon housing, all animals were drenched with 1ml $5kg^{-1}$ liveweight (LW) of Arrest combination drench (37.5g l^{-1} levamisole and 23.8g l^{-1} albendazole, Ancare New Zealand Ltd, Auckland, New Zealand). They were allocated hierarchically by liveweight (mean 26.0 kg ± 1.7) into one of four treatment groups (n=10). One group was infected with the abomasal parasite *Teladorsagia circumcincta* for 63 days (group IF); a second group received the same infection while concurrently treated once weekly with an intramuscular injection of 1ml $30kgLW^{-1}$ of the corticosteroid Depredone (40mg methylprednisolone acetate ml^{-1}, Jurox Pty. Ltd, Rutherford, NSW, Australia) in order to suppress immune function (group ISIF); the third group received the corticosteroid treatment only (group IS) while a fourth group remained as a control (group C), creating a 2 x 2 factorial design. This experiment was carried out with approval from, and in accordance with, the Lincoln University Animal Ethics Committee: approval No 31.

5.2.2. Management and measurements

All animals had access to fresh water and were offered, *ad libitum*, a complete ruminant diet, supplying an estimated 10.5 mega joules (MJ) of metabolizable energy (ME) and 68g of metabolizable protein (MP) kg dry matter $(DM)^{-1}$ (Table 5.1). Individual feed refusals were collected and weighed weekly. Sub-samples of feed offered and refused were taken for determination of dry matter (DM) after drying for 72h at 90°C. Liveweight was recorded at weekly intervals. Fasted LW (after 24h of food deprivation) was also measured on day 0 and on day 63 of infection to aid the estimation of body composition by computed tomography (described below). Blood samples were taken weekly from day 0 using jugular venipuncture into a 10ml vacutube (Becton Dickinson, VACUTAINER Systems, Rutherford, New Jersey, U.S.A) and stored at 4°C for

24h. After centrifugation at 2500rpm for 10min serum was separated and stored at –20°C. Slaughter of all infected animals and four from each of the C and IS groups occurred on day 63. Slaughter was carried out as described in Chapter 3 with liver and carcass weights recorded. At the conclusion of the trial, four IS and four C animals were housed in metabolism crates for 11 days for the determination of *in vivo* DM digestibility as described in Chapter 3.

Table 5.1: Composition and analyses of the complete ruminant diet (g $kgDM^{-1}$) that was offered *ad libitum* to all animals during the trial

	composition (g $kgDM^{-1}$)
Molasses	50
Barley	400
Oat Hulls	216
Broll	300
Urea	10
Sodium Phosphate	1.7
Dicalcium Phosphate	8
Potassium Carbonate	10.4
Magnesium Oxide	0.6
Salt	1.3
Mineral Mix†	2
Analysis	
CP (g $kgDM^{-1}$)	146
MJME $kgDM^{-1}$	10.5
MP supply (g $kgDM^{-1}$)	68
DTUP5‡	6.4
RP5§	108

NB. CP = crude protein, MJME = mega joules of metabolizable energy, MP = metabolizable protein

† 1kg contains: 5MIU vit A, 1MIU vit D, 7500IU vit E, 1g Co, 1g I, 20g Mn, 26g Fe, 25g Zn, 210g Mg, 4g P, 0·25g Se, 560g Ca

‡ Estimated digestible true undegraded protein at 5% rumen outflow rate (Agriculture and Food Research Council (AFRC), 1993)

§ Estimated rumen degradable protein at 5% rumen outflow rate (AFRC, 1993)

5.2.3. Parasitology

Animals were trickle-infected with the equivalent of 4000 L3 infective *T. circumcincta* larvae d^{-1} (130 L3 larvae kg initial LW^{-1}, Lincoln Kumeroa strain PB252/14) in a three-times-weekly dosing regime from day 2 until day 58 as described in Chapter 3. Weekly faecal samples were taken directly from the rectum for the determination of faecal nematode egg concentration (FEC; eggs $gram^{-1}$ (epg)) as described in Chapter 3. The remainder of the faecal sample not required for FEC was used to estimate faecal DM % from day 21 after drying for 72 h at 90°C. Lambs were fasted for 24h before slaughter. For each infected animal slaughtered the abomasum was ligated and excised, with care taken to ensure all gut contents were collected. Abomasal tissue was rinsed, with all washings collected before digestion for 12 h at 38.5°C in a 1% pepsin/HCL solution (Herlich, 1956). Sub-sampling and storage of both gut contents and digested tissue were carried out as described for the small intestine in Chapter 3. Counting and worm length measurements were as described in Chapter 3.

5.2.4. Serum analysis

Analysis of serum total protein, albumin and urea were determined using a Cobas Mira Plus Auto-analyser (Roche Diagnostics GmbH, Mannheim, Germany) as described in Chapter 3, with serum globulin levels calculated by difference. Serum *T. circumcincta*-specific L3 IgA and L3 total antibody measurements were analysed as described for *T. colubriformis* L3 IgA and total antibody in Chapter 3. Antigen coated plates were sourced from R.S. Green, AgResearch, Wallaceville, New Zealand. Briefly, plates were coated with 100µl of 1.2µg ml^{-1} *T. circumcincta* antigen $well^{-1}$ and then blocked with 5% skim milk powder in coating buffer. The following conjugated antibodies were utilized for each test: for L3 IgA, HRP conjugated rabbit anti-sheep IgA (1.0mg ml^{-1}, Bethyl Laboratories Inc, cat #A130-108P, lot #A130-108P-6) and for L3 total antibody HRP conjugated polyclonal rabbit anti-sheep immunoglobulins (DakoCytomation, Denmark, cat #091031, lot #00003906). Colour was developed for 40 and 12 min for L3 IgA and L3 total antibody, respectively.

5.2.5. Carcass composition

Changes in the bone, muscle and fat content of the carcass were estimated *in vivo* using x-ray computed tomography on days –2 and 62 of infection. Sedation, restraint and scanning procedure were as described in Chapter 3. Briefly, three anatomical reference x-ray cross sections were taken from each animal at the thoracic vertebrae 8, lumbar vertebrae 5 and ischium. In addition, five animals were randomly selected on each occasion for estimation of total carcass tissue weight using the calvaleri principle of Gundersen and Jensen (1987). Calvaleri estimated carcass weight (CW) was corrected to actual CW obtained from slaughtered animals. Carcass weight of the slaughtered animals was regressed with fasted LW prior to slaughter (*CW = 0.5866 X fasted LW - 2.8294;* R^2*=0.92*) which was then utilized to estimate the carcass weight of animals on day -2 and those that were not slaughtered on day 63. The weight of bone was estimated from carcass weight, based on the measurements of Fourie *et al*. (1970) (*bone wt (kg) = 0.2491 X* $CW^{0.7321}$) and subtracted from the carcass weight to give bone-free carcass weight. Proportions of fat and muscle in the bone-free carcass weight were estimated from the three reference slices. Proportions of fat and muscle in the reference slices were corrected to proportions of fat and muscle in the bone-free carcass in those animals that underwent a calvaleri scan using the following equations: *calvaleri fat % in the bone free carcass = 1.3267 X fat % in the bone free reference slices – 1.496 (*R^2 *= 0.88); calvaleri muscle % in the bone free carcass = 0.846 X muscle % in the reference slices + 1.4656 (*R^2 *= 0.93)*. The net energy (NE) deposited in the carcass gain was calculated assuming that muscle consisted of 0.20 protein, using energy values of 38.9 and 22.2 mega joules (MJ) kg^{-1} for fat and protein, respectively (Blaxter and Rook, 1953).

5.2.6. Wool production

Animals were shorn on days -5 and 63, and greasy fleece weight recorded on the latter date used as a measure of total wool production. A sub-sample of mid-side fleece wool was taken from each animal on day 63 and scoured to estimate yield of clean fleece weight by repeated plunging in water at 60°C containing 1ml l^{-1} Teric GN-9 (ICI, Australia) before washing in clean water and drying at 60°C in a forced air oven as described in Chapter 4. Energy deposition in the fleece was calculated assuming 23.7MJ kg^{-1} clean fleece weight (AFRC, 1993).

5.2.7. Abomasal pH

The pH of abomasal contents of all slaughtered animals was measured after the ligation and removal of the abomasum using a Shindengen KS701 pH BOY ISFET pH meter (Total Lab Systems Ltd, Auckland, New Zealand).

5.2.8. Tissue biopsy at slaughter

For each slaughtered animal, one tissue sample intended for mucosal mast cell, globule leukocyte and eosinophil counts was collected immediately from both the abomasum and small intestine (1m distal to the pylorus). Tissues were fixed in 4ml of 4% paraformaldehyde (4g paraformaldehyde $100ml^{-1}$ freshly prepared phosphate buffered saline (PBS); pH 7.4) for 6h at room temperature before being transferred to 1.5 ml of 70% ethanol until processing.

Immunological cell counts of terminal biopsy samples were performed by J.F. Huntley, Moredun Research Institute, Scotland, according to the methods outlined by Huntley *et al*. (1995). Briefly, mucosal mast cells were counted after embedding in paraffin wax and slicing at 5µm intervals before staining with toluidine blue (pH 0.5). Stained cells were counted under a X 12.5 eye piece containing a calibrated graticule and X 25 objective lens that provided a viewing area of 0.465mm^2 per graticule. Eosinophils and globule leukocytes were differentiated on morphological criteria after staining with carbol

cromotrope and counted under a X 12.5 eye piece containing a calibrated graticule and a X 40 objective lens that provided and area of 0.29mm^2 per graticule. Cell counts were recorded in 10 graticule fields and expressed as cells per 0.2mm^2.

5.2.9. Cannulated animals

In addition to the trial described above, further information on the immunological changes occurring at the local site of infection was sought using cannulated animals. Twelve parasite-naïve four-and-a-half-month old ewe lambs that were sourced from the same pool of animals utilized for the trial described above were surgically fitted with an abomasal cannula by A.S. Familton. Briefly, animals were fasted for 24h and removed from water for 12h prior to surgery. Anaesthesia was induced by combined intravenous administration of 0.75mg kg^{-1}, diazepam 5mg ml^{-1}, (Pamlin, Parnell Lab NZ, Auckland) and 15mg kg^{-1} Ketamine HCl 100mg ml^{-1} (Phoenix Ketamine Injection, Phoenix Pharm Distributors Ltd, New Zealand). After clipping the flank a vertical incision was made between the hindmost rib and the hipbone approximately 15cm from the lumbar vertebrae, from which the abdominal muscle wall was separated by blunt dissection. The abomasum was located and exteriorised and held in place with Allis forceps while a purse string suture was placed around the area where the cannula was to be inserted. An abomasal cannula with internal flange (shown in Plate 1) was inserted through an incision approximately 10cm anterior to the pylorus and the purse string suture then tightened and tied off. The abomasum was returned to the abdominal cavity and exteriorised through a stab incision made between the two hindmost ribs and held in place by the external flange and rubber retaining clip (see Plate 1). Animals were allowed to recover for six weeks before being allocated hierarchically by liveweight (mean 26.9 kg ± 1.74) into one of two treatment groups; cannulated and infected (CIF; n=6), or cannulated and immuno-suppressed and infected (CISIF; n=6). Feeding, treatment and sampling of CIF and CISIF animals during experimentation was identical to that described for IF

and ISIF animals, respectively, with the exceptions that changes in carcass composition and wool production were not measured and infection was extended until slaughter at day 91. The cannulation of animals and experimental procedure was carried out with approval from, and in accordance with, the Lincoln University Animal Ethics Committee: approval No 16.

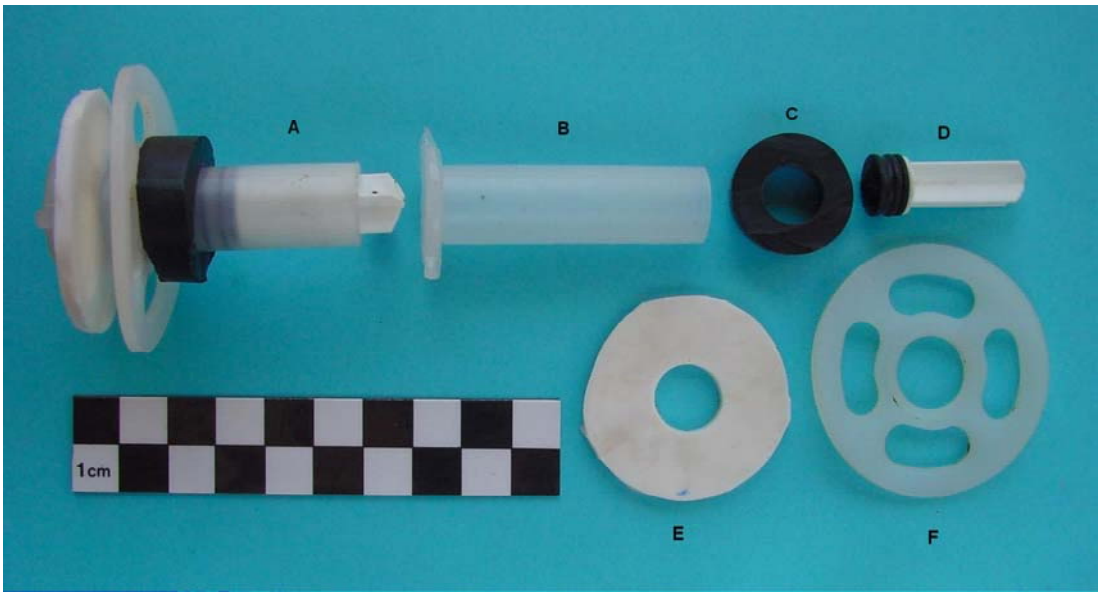

Plate 5.1: Abomasal cannula surgically fitted to cannulated animals. A) entire cannula as fitted, B) cannula barrel, C) rubber retaining clip, D) plunger, E) internal flange, F) external flange. Photo courtesy of M. R. Ludemann.

5.2.10. Serial biopsy sampling

Abomasal pH measurements and serial abomasal tissue biopsy samples were taken from cannulated animals at weekly intervals from day -1. At each occasion, abomasal fluid was collected upon opening of the cannula and pH measured immediately using a Shindengen KS701 pH BOY ISFET pH meter (Total Lab Systems Ltd, Auckland, New Zealand). Two abomasal tissue samples were taken using mucosal biopsy forceps (CE 0124, Richard Wolf, GmBH) as described by Huntley *et al.* (2004). One serial biopsy sample was intended for mucosal mast cell, globule leukocyte and eosinophil counts and the second intended for the measurement of tissue antibody. Fixation and storage for the former was carried out as previously described for the terminal

biopsy samples of non-cannulated animals; the latter sample was placed at immediately at -20°C until processing. As the exact location from which serial tissue samples were collected and homogeneity of tissue antibody within the abomasum was not known, one tissue sample for antibody analysis was collected at slaughter from each cannulated animal from both the fundic and pyloric ends of the abomasum and stored at -20°C until processing. In addition, abomasal pH measurements and terminal biopsy samples were taken at slaughter from all cannulated animals as described previously for non-cannulated animals.

Staining and counting of cell types was carried out by J.F. Huntley as described previously for non-cannulated animals. As the size and suitability of the serial biopsy samples varied, counts of cells in up to 10 graticule fields were recorded, with cell counts expressed as cells per 0.2mm^2.

5.2.11. Tissue antibody

Tissue antibody analyses of serial and terminal samples were performed by J.F. Huntley. Tissue antibody levels were determined from a homogenous sample utilizing a double-binding sandwich ELISA for *T. circumcincta* specific L3 antibodies as previously described for serum. Briefly, tissues were defrosted and weighed before the addition of 500µl of PBS/tween 20 wash buffer then ribulysed at speed 4.5 for 40 sec. After resting on ice for 5 min, samples were centrifuged for 5 min at 14,000 rpm, the supernatant then removed and stored at -20°C until analysis for parasite specific L3 IgE, IgG and IgA. Tissue antibody results were corrected for background absorbance and expressed as mean optical density (OD) mg^{-1} of tissue.

5.2.12. Statistical analysis

Data were analysed using GENSTAT statistical package (Lawes Agricultural Trust, 2003) and are presented as group means unless otherwise stated. FEC and worm burdens were log transformed (log10 (count + 1)) before analysis. Tissue biopsy cell counts were square-root transformed (Sqrt (count + 1)) with analysis weighted by the number of measurements obtained from each sample. For all transformed data, back-transformed means are presented. Diet digestibility, worm burdens, worm length, worm sex ratio, liver weight, carcass composition and wool production were analysed using a general analysis of variance (ANOVA). All remaining measures underwent sequential comparison of ante-dependence structures for repeated measures before being analysed using restricted maximum likelihood (REML) with the standard error of the difference calculated and used to compare individual groups using a one-tailed t-test. One animal from group C (control) was an outlier, with a consistently low intake and liveweight, and was accounted for by blocking during analysis. Data from cannulated animals was analysed separately from those of the non-cannulated animals. Serial biopsy tissue samples on one day (day 63) appeared abnormal, with markedly reduced mast cells and globule leukocytes compared with the previous and subsequent biopsy samples. Consequently the cell counts of tissue samples from this day were excluded from statistical analysis, with the values in question displayed separately.

5.3. Results

No clinical signs of parasitism were observed in any of the infected animals at any stage. The cannulae of three CIF animals caused inflammation in the abomasal mucosa and consequently closed over from day 77; as a consequence, these animals were slaughtered on day 85. The remaining CIF and all CISIF animals were slaughtered on day 91.

Digestibility of the feed offered was not affected by corticosteroid treatment, being 63.6 ± 2.1 and 62.1 ± 3.9 for treated and non-treated animals, respectively, ($p=0.26$).

Liver weights at slaughter of non-cannulated animals averaged 624 ± 94, 561 ± 78, 727 ± 57, and 690 ± 107 g for C, IF, IS, and ISIF, respectively, and were increased by immuno-suppression ($p=0.001$) and tended to be decreased, but not significantly so by infection ($p=0.196$). Liver weights of cannulated animals were significantly affected by treatment, averaging 439 ± 51 and 674 ± 68 g in CIF and CISIF, respectively, ($p<0.001$).

5.3.1. Faecal dry matter

Mean DM percentage of faeces are shown in Figure 5.1. Amongst non-cannulated animals there was a significant infection x time interaction (p=0.02) reflected in a decrease in IF animals from an initially elevated level to a value similar to those of the other groups from day 35. Neither immuno-suppression alone (IS), nor infection in addition to immuno-suppression (ISIF) affected faecal DM concentration at any stage (p>0.05). Amongst cannulated animals, there was an overall effect of immuno-suppression, with CISIF animals having, on average a 7% lower faecal DM than CIF (p=0.03). This was primarily due to CIF being proportionately 0.21 greater than CISIF on days 21 and 28 (p=0.02), a difference that abated from day 35 onwards. Despite this, there was no significant immuno-suppression x time interaction (p=0.54).

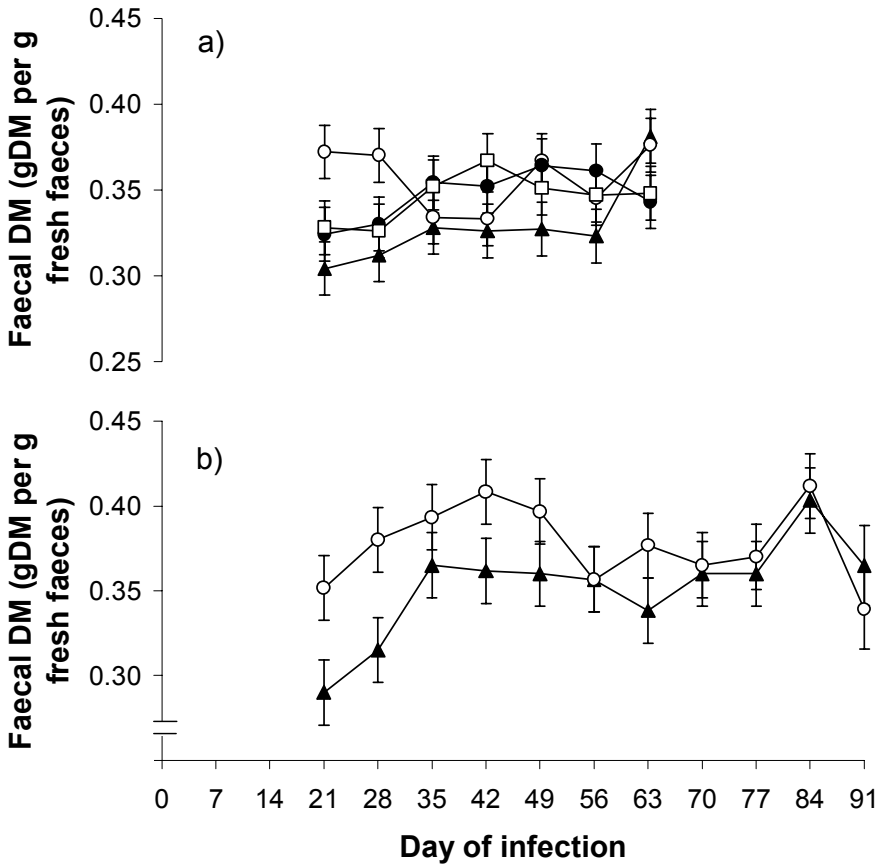

Figure 5.1: Mean dry matter percentage of faeces from a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

5.3.2. Feed intake

Mean feed intakes are shown in Figure 5.2. Non-cannulated animals displayed an immuno-suppression x time interaction ($p<0.01$) since immuno-suppression increased feed intake compared with both C and IF sheep regardless of concurrent infection, a difference that abated with time. Infection alone (IF) tended to reduce intake, with significant proportionate reductions compared with C animals of 0.17 observed on days 21 and 28 ($p=0.04$ and 0.05, respectively). In cannulated animals, there was an effect of immuno-suppression ($p=0.001$) and time ($p=0.001$), but no immuno-suppression x time interaction ($p=0.74$). This was reflected in a 0.23-0.46 lower intake in CIF relative to CISIF throughout the trial even as intakes of CIF animals rose to 1.50 times of their initial values on day 21 and on day 63.

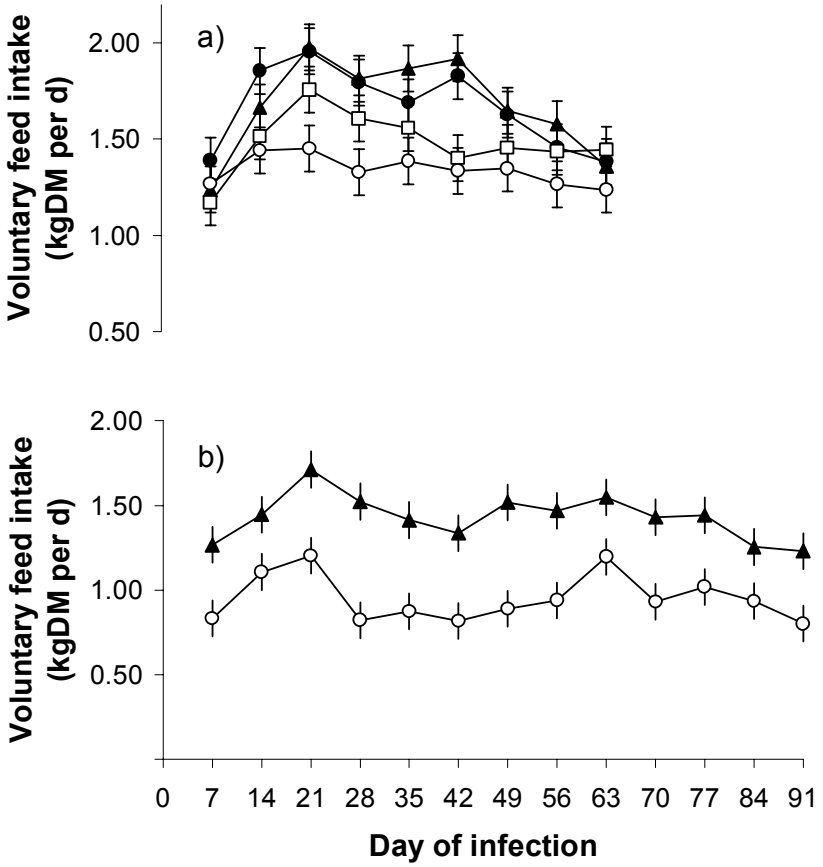

Figure 5.2: Mean voluntary feed intake (kgDM d^{-1}) of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

5.3.3. Liveweight

Mean liveweights during the trial are shown in Figure 5.3. Amongst non-cannulated animals, there was an increase in the LW of all groups with time ($p=0.01$). There tended to be an effect of immuno-suppression ($p=0.066$) and a suppression x time interaction ($p=0.07$) reflecting comparable increases in IS and ISIF that were greater than in non-suppressed animals. The LW of group IF was impaired from day 22 ($p<0.04$), resulting in IF animals having a 0.11 proportionate lower fasted LW than C animals at day 63 ($p=0.01$). Values for both IS and ISIF were similar to C animals throughout. Amongst cannulated animals, there was an immuno-suppression x time interaction ($p=0.003$) due to an increasing difference in LW until day 42, resulting in CISIF animals having a 0.18 relatively heavier fasted liveweight on day 91 ($p=0.001$).

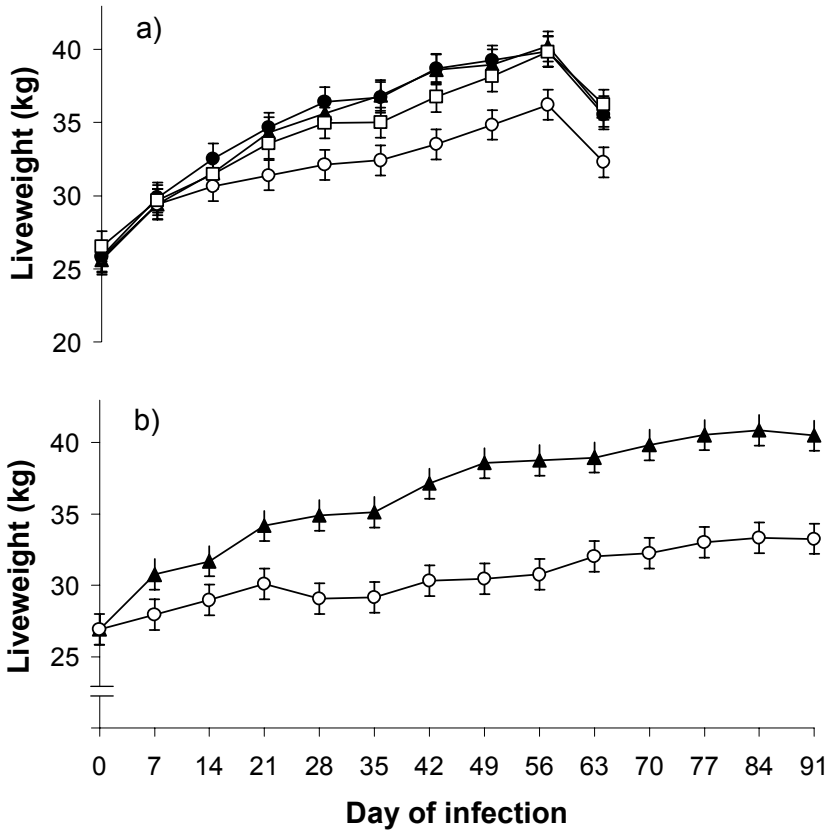

Figure 5.3: Mean liveweight of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C). Values are 24h fasted liveweight for non-cannulated on days 0 and 63 and for cannulated animals on days 0 and 91.

5.3.4. Faecal egg counts

Mean FEC are given in Figure 5.4. Eggs were not detected in the faeces of C or IS animals at any stage. There was a highly significant infection x immuno-suppression x time interaction ($p=0.001$) in non-cannulated animals. This reflected an increase in all infected animals from day 21. The peak FEC in HIF animals was on day 28 which subsequently declined to less than 50 epg from day 35. In ISIF animals, FEC continued to rise to a plateau of about 1,600epg from day 42. In cannulated animals, there was an immuno-suppression x time interaction ($p=0.001$). FEC in CIF and CISIF were similar until day 28, after which a reduction in counts was observed in CIF animals, while counts in CISIF continued to increase to in excess of 2,000 epg. However, while counts in CIF had fallen to as low as 16 epg on day 49, they continued to fluctuate, and eventually increased to 521 epg on day 91, a level that was not significantly different to CISIF ($p=0.08$).

Total daily egg production is shown in Figure 5.5. Patterns of egg excretion were similar to those observed for FEC. There was an infection x immuno-suppression x time interaction ($p<0.01$) for non-cannulated and an immuno-suppression x time interaction for cannulated animals ($p<0.001$). Total egg excretion from both IF and CIF animals peaked at 110,000 eggs d^{-1} on day 28, with rates of excretion from ISIF and CISIF animals varying between 250,000 and 420,000 eggs d^{-1} from day 42, levels which were significantly greater than those in their immunologically normal contemporaries ($p<0.01$) with the exception of the difference between cannulated animals on day 91 ($p=0.12$).

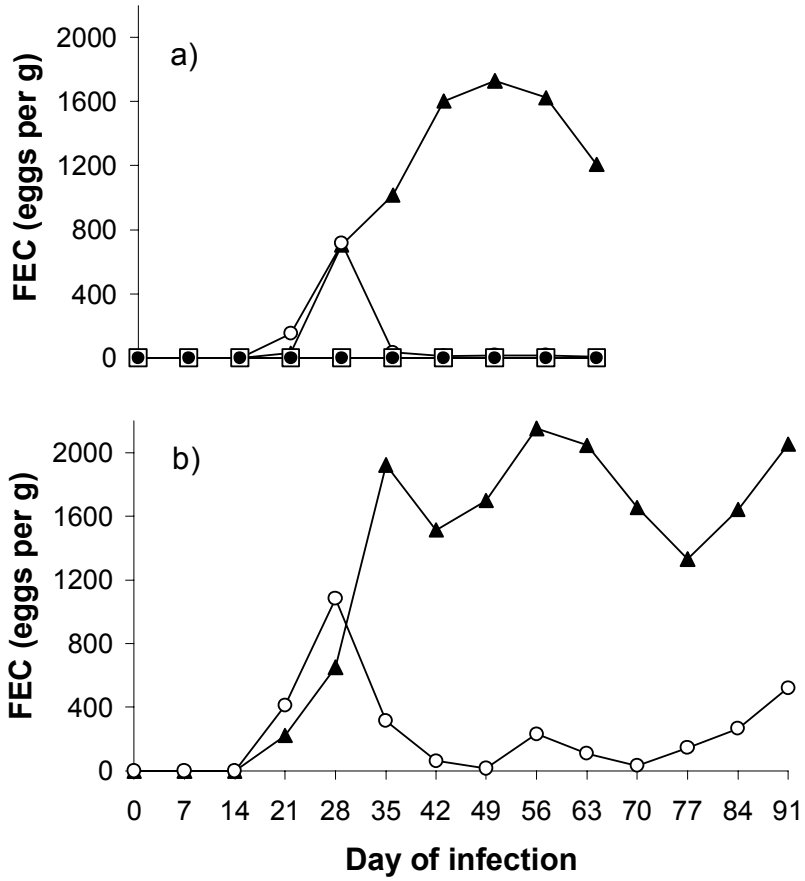

Figure 5.4: Mean back-transformed (log10 (count + 1)) faecal egg counts (FEC) of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

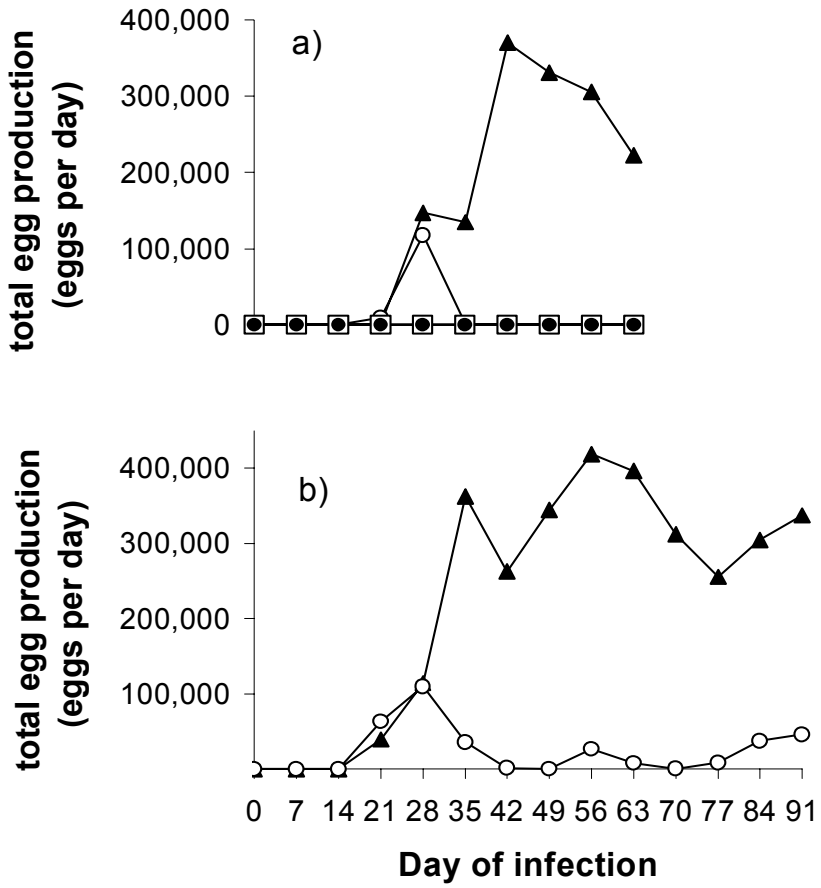

Figure 5.5: Mean back-transformed (log10 (count + 1)) total daily faecal egg excretion of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

5.3.5. Worm burden and worm length

Mean worm burdens at slaughter for all infected non-cannulated animals are given in Table 5.2. Immuno-suppression had no effect on L3 larval numbers ($p=0.15$), but did result in larger populations of both L4 larvae ($p<0.001$) and adult L5 worms ($p<0.001$). Consequently, IF animals had a total worm burden that was only 0.15 that of ISIF animals ($p<0.001$). Immuno-suppression increased the proportion of L4 larvae that were inhibited from 0.11 in IF to 0.68 in ISIF ($p<0.001$). In addition, immuno-suppression reduced the proportion of L5 adult nematodes that were female from 0.71 in IF to 0.59 in ISIF ($p=0.02$). Male adult worms were found to be relatively 0.24 shorter than females ($p<0.001$) regardless of immuno-suppressive treatment. Immuno-suppression did not affect the combined mean worm length of adult male and female worms, being 8.49 ± 3.98 and 8.13 ± 1.83 mm for IF and ISIF, respectively, ($p=0.487$).

Mean worm burdens at slaughter of the cannulated animals are given in Table 5.3. Like their non-cannulated counterparts, cannulated animals that were immuno-suppressed (CISIF) had similar numbers of L3 larvae ($p=0.25$) and greater numbers of L4 larvae ($p<0.001$) and L5 adult worms ($p=0.002$) than their CIF counterparts. Also, similar to their non-cannulated counterparts, CIF had a total burden that was 0.16 of that found in CISIF animals ($p<0.001$). Immuno-suppression increased the proportion of L4 larvae that were inhibited from 0.39 in CIF to 0.75 in CISIF ($p=0.007$), and tended to reduce the proportion of L5 adults that were female from 0.64 in CIF to 0.55 in CISIF ($p=0.08$). Regardless of immuno-suppression, mean adult male worm length was comparatively 0.22 less than female length ($p<0.001$), with the combined average length of adult male and females tending to be longer in CISIF compared with CIF animals, being 8.09 ± 1.43 and 8.43 ± 1.46 mm for CIF and CISIF, respectively, ($p=0.051$).

Table 5.2: Numbers of log10 (count + 1) worms and stage of development (L3, L4 or L5 adult) recovered from non-cannulated animals at slaughter infected with 4000 *T. circumcincta* d^{-1} (IF) or similarly infected and immuno-suppressed (ISIF). Back transformed means are given in parenthesis.

	IF	ISIF	s.e.m
L3	1.62[a] (42)	2.46[a] (288)	0.395
L4	3.05[a] (1,132)	3.96[b] (9,099)	1.220
L5 Adult	3.64[a] (4,365)	4.46[b] (28,840)	0.144
Total	3.77[a] (5,929)	4.60[b] (39,994)	0.135

[a,b] Within each stage of larval development, values with different superscript are significantly different ($p<0.01$)

Table 5.3: Numbers of log10 (count + 1) worms and stage of development (L3, L4 or L5 adult) recovered from cannulated animals at slaughter infected with 4000 *T. circumcincta* d^{-1} (CIF) or similarly infected and immuno-suppressed (CISIF). Back transformed means are given in parenthesis.

	CIF	CISIF	s.e.m
L3	2.03[a] (107)	2.85[a] (708)	0.479
L4	3.03[a] (1,064)	4.17[b] (14,689)	0.101
L5 Adult	3.46[a] (2,877)	4.22[b] (16,596)	0.131
Total	3.73[a] (5,383)	4.54[b] (34,356)	0.092

[a,b] Within each stage of larval development, values with different superscript are significantly different ($p<0.01$)

5.3.6. Carcass composition, wool production and energy utilization

Computed tomography-estimated changes in carcass composition are shown in Table 5.4. Carcass composition of all groups at day -2 was similar. Infection alone proportionately reduced bone deposition by 0.37 (p=0.01) and muscle deposition by 0.46 (p=0.01), but did not affect fat deposition (p=0.17) nor wool production (p=0.25) compared with C animals. Immuno-suppression increased the relative fat deposition by 0.47 (p=0.03) and decreased muscle deposition by 0.35 (p=0.01) but did not affect bone growth (p=0.29) or wool production (p=0.35). Infection in addition to immuno-suppression did not result in any difference in tissue deposition between ISIF and IS animals, although wool production did tend to be comparatively reduced by 0.12, (p=0.07).

Infection alone tended to reduce net energy (NE) deposition, with IF having a 0.24 proportionate lower NE deposition than C animals (p=0.11). Immuno-suppression alone tended to increase NE deposition, with IS having a 0.29 greater relative NE deposition than C animals (p=0.07). Infection in addition to immuno-suppression had no effect on NE deposition (p=0.42). In comparison to C animals, total ME intake tended to be reduced by 0.10 in IF animals (p=0.10) and tended to be increased by 0.11 in IS animals (p=0.08). Infection in addition to immuno-suppression had no effect on total ME intake (p=0.46). Gross efficiency of utilization of ME tended to be proportionately reduced by 0.20 in IF compared to C animals (p=0.07), and was not affected by immuno-suppression (p=0.15), or infection in addition to immuno-suppression (p=0.22).

Table 5.4: Computed tomographically estimated carcass growth, wool production and energy utilization of animals while infected with 4000 *T. circumcincta* d^{-1} (IF), similarly infected and immuno-suppressed (ISIF), immuno-suppressed only (IS) or maintained as controls (C).

	C	IF	IS	ISIF	s.e.m
Original composition (day -2)					
Liveweight (kg)	26.0	26.0	26.1	25.8	0.57
Carcass weight (kg)	12.4	12.4	12.5	12.3	0.34
Bone weight (kg)	1.59	1.57	1.58	1.57	0.03
Muscle weight (kg)	7.49	7.81	7.77	7.56	0.23
Fat weight (kg)	3.59	2.87	3.06	3.22	0.30
Tissue deposition (day 62)					
Bone deposition (kg)	0.54^{a}	0.34^{b}	0.50^{a}	0.52^{a}	0.06
Muscle deposition (kg)	3.26^{a}	1.76^{b}	2.12^{b}	2.39^{b}	0.36
Fat deposition (kg)	2.41^{a}	1.85^{a}	3.54^{b}	3.43^{b}	0.41
Clean wool wt (kg)	0.79^{a}	0.75^{ab}	0.77^{ab}	0.68^{b}	0.04
Energy Utilization†					
Total ME intake (MJ)	868^{ab}	779^{a}	967^{b}	974^{b}	48.8
Total NE deposited (MJ)	134^{ab}	102^{a}	172^{b}	167^{b}	17.8
NE:ME	0.15^{ab}	0.12^{a}	0.17^{b}	0.17^{b}	0.01

[a,b]Within each row, values with different superscripts are significantly different ($p<0.05$).

† ME = meatbolizable energy, NE = net energy.

5.3.7. Serum total protein

Mean serum total protein concentrations are shown in Figure 5.6. Amongst non-cannulated animals, immuno-suppressed groups tended to have greater total protein concentrations ($p=0.048$). There was also an effect of time ($p<0.001$) reflected in an increase in all groups throughout the trial. In addition, there tended to be an infection x immuno-suppression x time interaction ($p=0.069$) reflected in serum total protein concentrations in IF animals increasing at a slower rate than in C, IS and ISIF animals until day 42. Amongst cannulated animals there were effects of immuno-suppression ($p<0.001$) and time ($p<0.001$). The latter was reflected in an overall increase in total protein in both CIF and CISIF throughout the trial, and the former due to a higher concentration in CISIF from day 21.

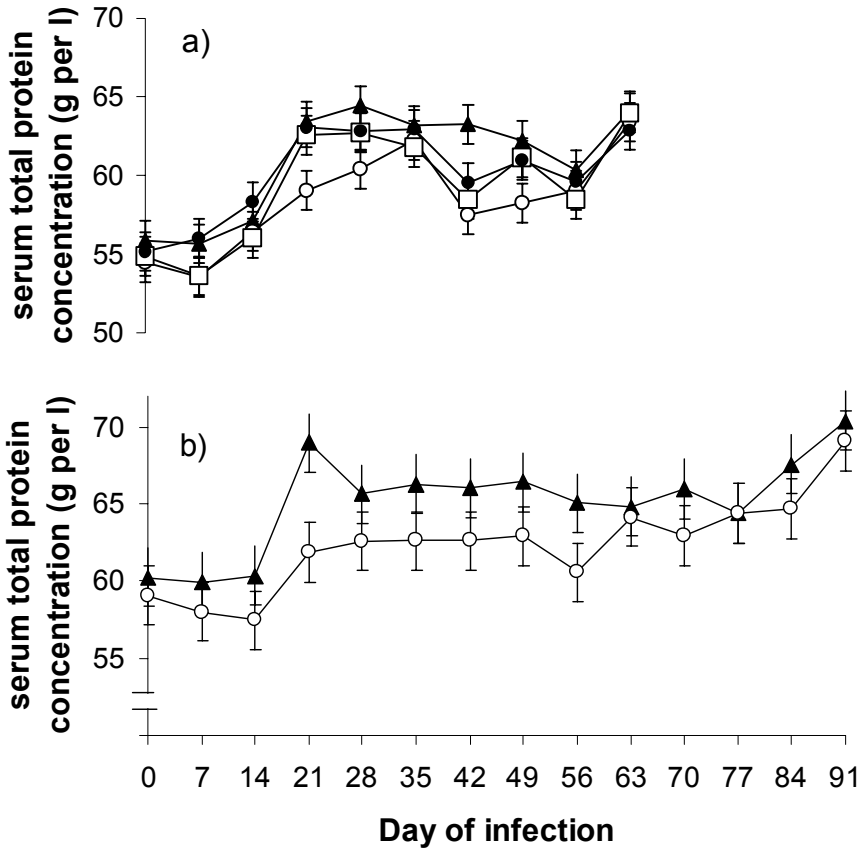

Figure 5.6: Mean serum total protein concentration of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

5.3.8. Serum albumin

Mean serum albumin concentrations are given in Figure 5.7. Serum albumin concentrations increased with time ($p=0.001$) while being reduced due to infection ($p=0.034$) and increased through immuno-suppression ($p=0.008$). Animals that were concurrently infected and immuno-suppressed did not suffer any reduction in albumin concentration ($p>0.05$). Amongst cannulated animals there was an immuno-suppression x time interaction ($p<0.001$) since CIF experienced a depression of albumin concentration compared with CISIF from day 14 that increased in magnitude to a peak proportionate reduction of 0.24 at day 84.

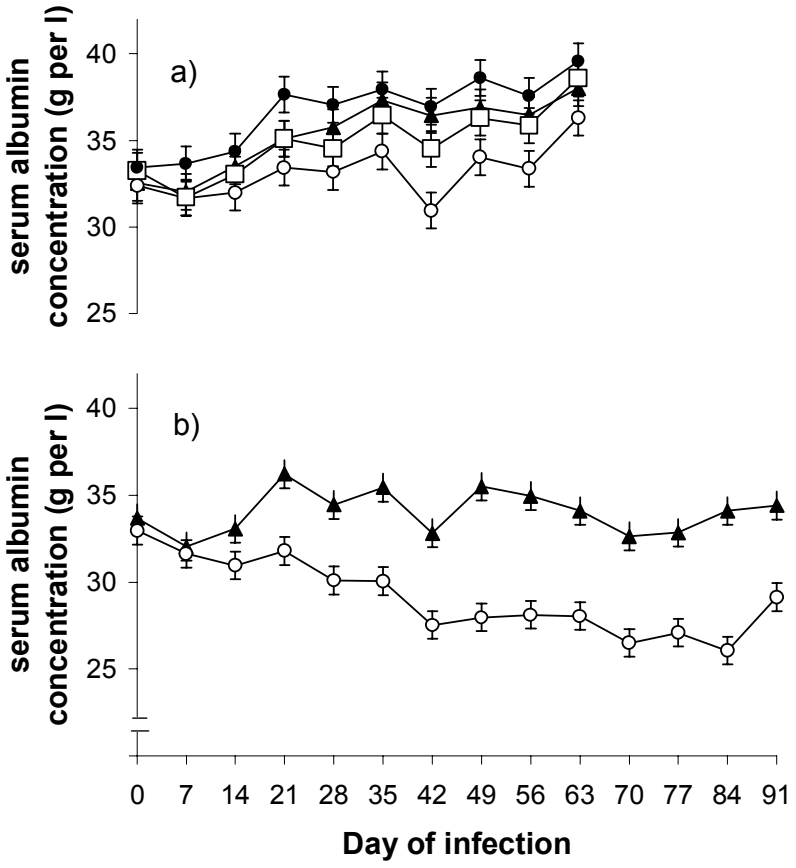

Figure 5.7: Mean serum albumin concentrations of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

5.3.9. Serum globulin

Mean serum globulin concentrations are given in Figure 5.8. Overall, there was an infection x immuno-suppression x time interaction ($p<0.001$) in non-cannulated animals reflecting an increase in both infected groups (from day 35 and 21 for IF and ISIF, respectively) while immuno-suppression alone (IS) tended to reduce concentrations. In cannulated animals, there was an effect of time ($p<0.001$), reflecting an increase in both groups throughout the trial, and there tended to be an effect of immuno-suppression ($p=0.052$) with CIF having greater concentrations than CISIF.

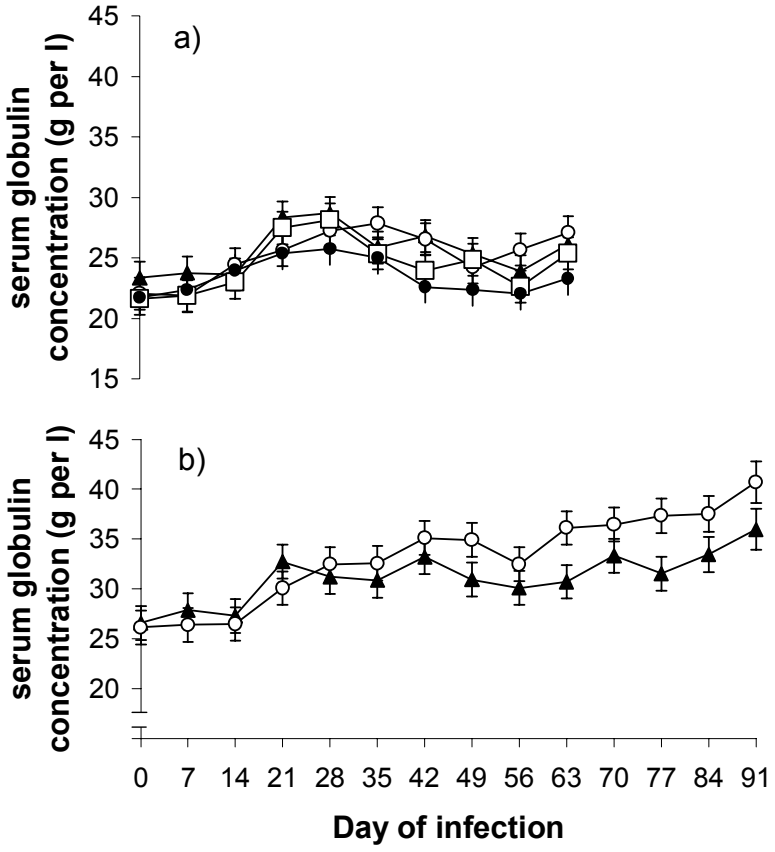

Figure 5.8: Mean serum globulin concentration of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

5.3.10. Serum urea

Mean serum urea concentrations are given in Figure 5.9. Urea concentrations increased in all groups during the trial ($p<0.001$). Also, there tended to be an immuno-suppression x infection interaction ($p=0.077$) reflecting in lower concentrations in IF than in those that were concurrently immuno-suppressed (ISIF). Amongst cannulated animals there were overall effects of time and immuno-suppression ($p=0.001$ in both cases). This was reflected in an increase and then a decrease in serum urea concentrations in both groups with time and maintenance of levels relatively 0.23 to 0.38 greater in CISIF than CIF from day 28 ($p<0.04$ in all cases at all times).

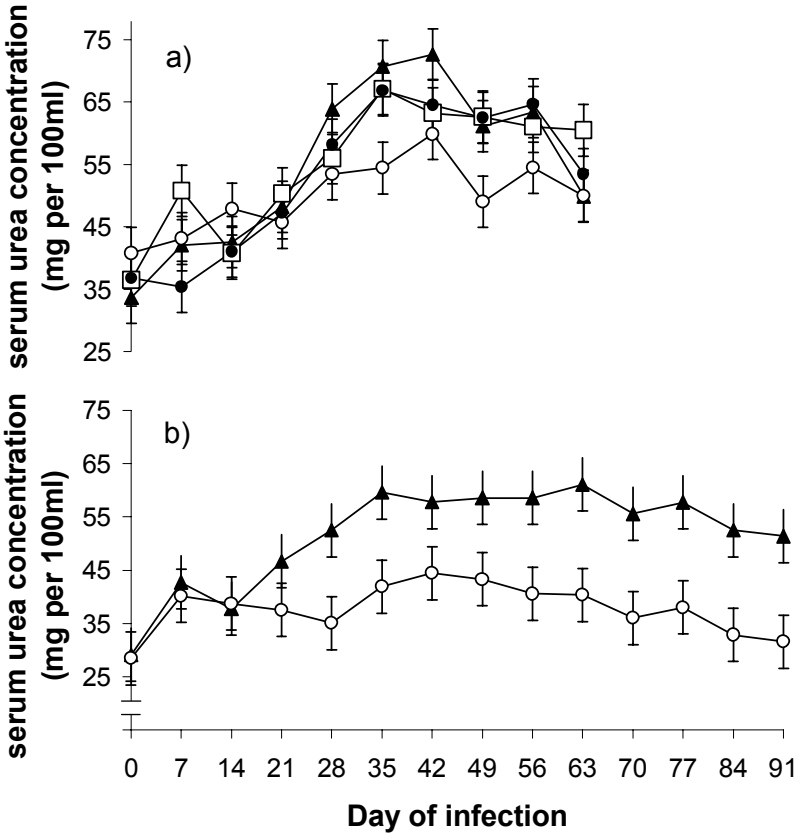

Figure 5.9: Mean serum urea concentrations of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

5.3.11. Serum L3 total antibody

Mean *T. circumcincta* specific total antibody absorbance is given in Figure 5.10. Overall, there was a immuno-suppression x infection x time interaction ($p<0.001$) reflecting an increase in both infected groups, regardless of immuno-suppression status, until day 35 which then subsided in ISIF but remained elevated in IF animals. In addition, ISIF animals were found to have 0.15 proportionate increase in pre-infection total antibody levels at day 0 ($p=0.05$). There was an immuno-suppression x time interaction in cannulated animals ($p<0.001$) that was reflected in an increase in all animals until day 42 which was maintained in CISIF but subsided in CIF animals.

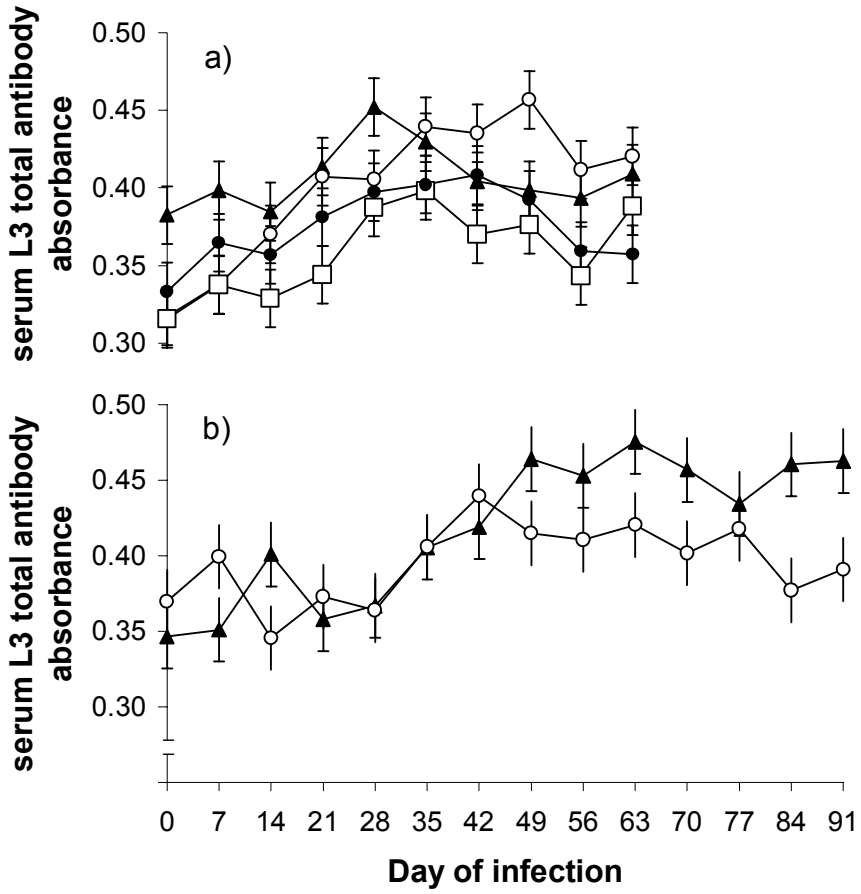

Figure 5.10: Mean serum total *T circumcincta* L3 specific antibody absorbance of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

5.3.12. Serum L3 IgA

Mean *T. circumcincta* specific serum L3 IgA absorbance is shown in Figure 5.11. There was a significant infection x immuno-suppression x time interaction ($p<0.001$) reflecting a proportionate increase of 0.21 in IF and 0.44 in ISIF compared with C and IS animals, respectively, from days 35 to 56 ($p<0.02$), which then abated to only 0.08 on day 63 ($p=0.13$). Amongst cannulated animals there was an immuno-suppression x time interaction ($p=0.026$) since levels in both groups increased with time but at a greater rate in CISIF from day 35 to 49.

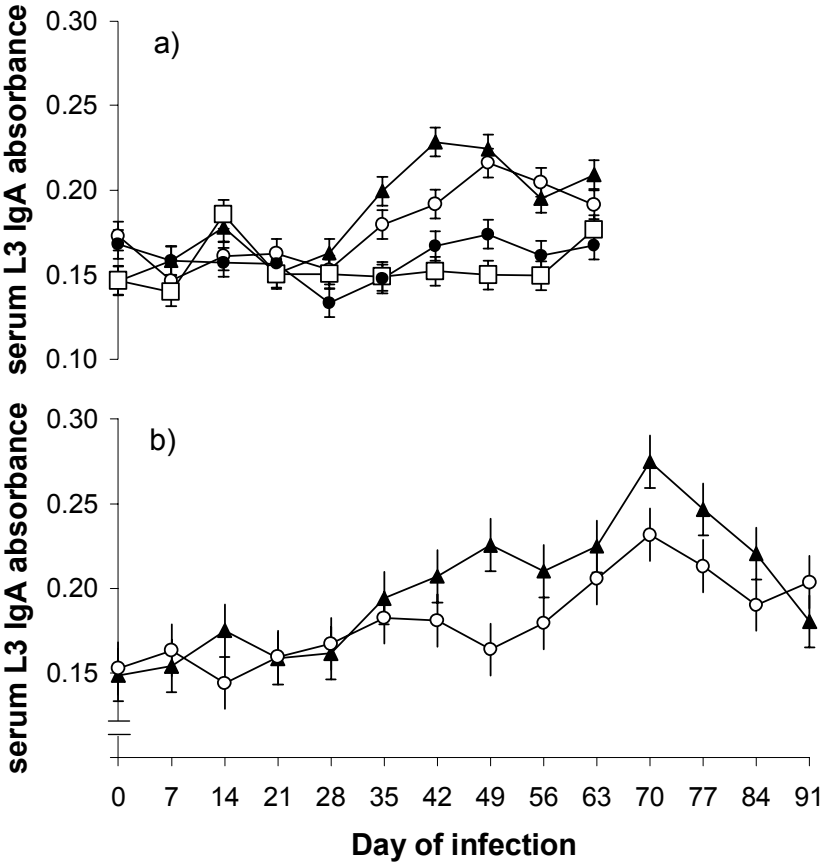

Figure 5.11: Mean serum *T. circumcincta* L3 specific IgA of a) non-cannulated or b) cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (IF, CIF), ▲ similarly infected and immuno-suppressed (ISIF, CISIF), ● immuno-suppressed only (IS) or □ maintained as controls (C).

5.3.13. Abomasal pH

Abomasal pH at slaughter of non-cannulated animals averaged 3.89 ± 0.71, 2.38 ± 0.26, 2.60 ± 0.22 and 4.17 ± 1.04 for IF, IS, C and ISIF animals, respectively, and was affected by infection ($p<0.001$) but not immuno-suppression ($p=0.65$). Serial abomasal pH measurements from cannulated animals are shown in Figure 5.12. Overall there was an immuno-suppression x time interaction ($p=0.002$) reflecting no difference between CISIF and CIF until day 49, but thereafter, values being comparatively 0.24 to 0.37 greater in CIF than in CISIF with the exception of days 63 ($p=0.35$) and 84 ($p=0.17$). Abomasal pH at slaughter of cannulated animals averaged 5.35 ± 0.91 and 5.27 ± 0.86 for CISIF and CIF, respectively, values which were not different ($p=0.87$).

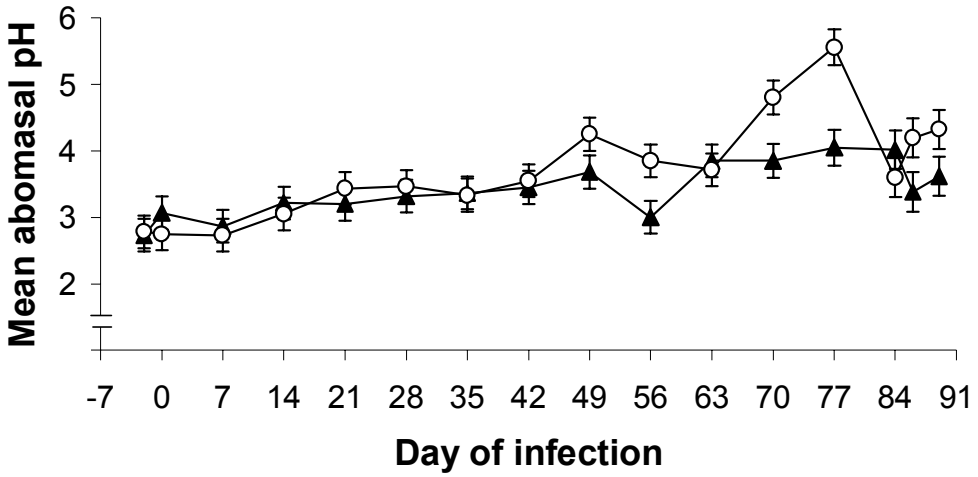

Figure 5.12: Mean pH of abomasal fluid in cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (CIF) or ▲ similarly infected and immuno-suppressed (CISIF).

5.3.14. Serial tissue antibody

Mean IgE, IgG and IgA absorbance of the serial biopsy tissue samples is shown in Figure 5.13. For IgE there was an effect of time ($p<0.001$) but not of immuno-suppression ($p=0.771$). For IgG there was an effect of time ($p<0.001$) but there was no effect of immuno-suppression ($p=0.872$). There was an immuno-suppression x time interaction for IgA ($p<0.001$), as only CIF displayed an increase from day 35 that was maintained during the remainder of the trial.

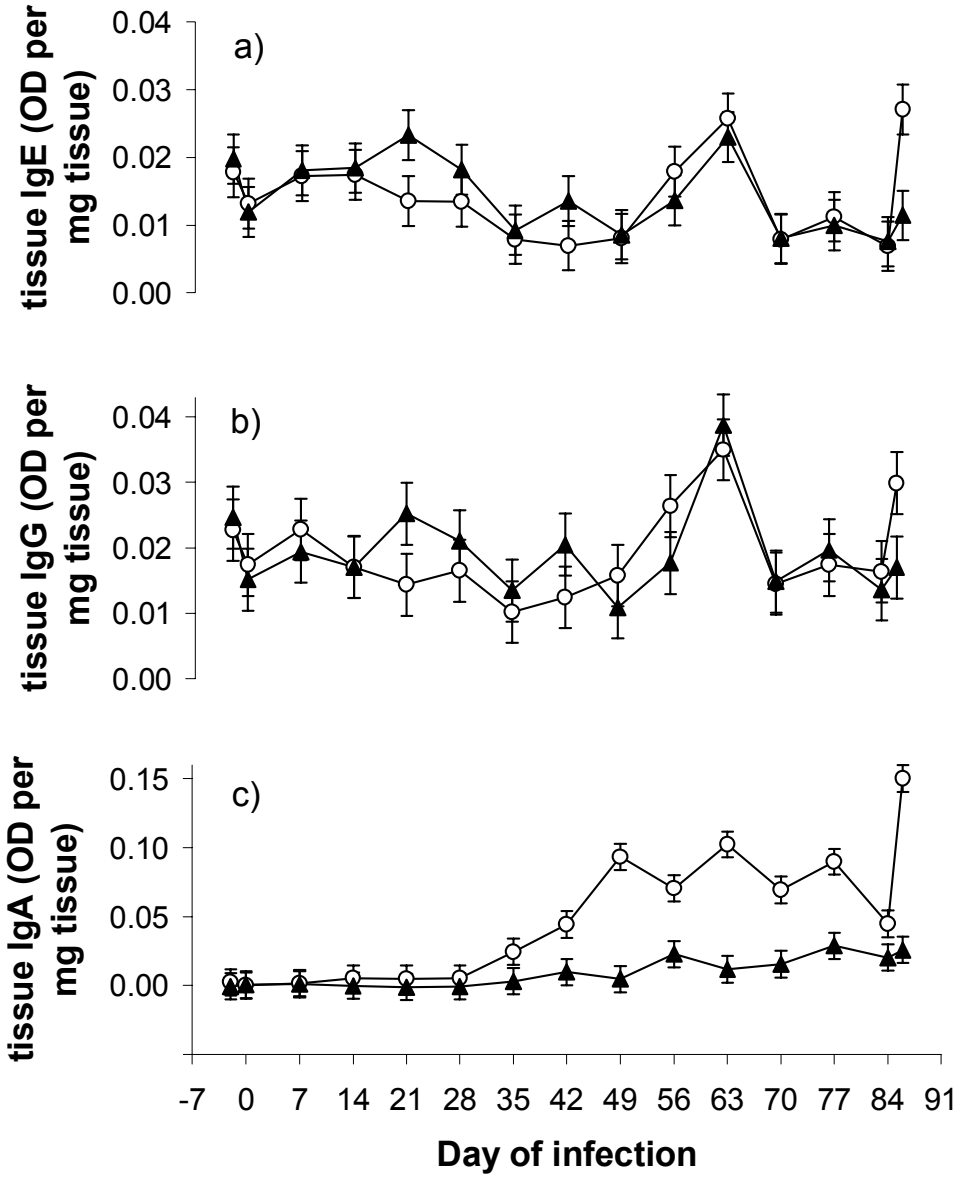

Figure 5.13: Mean serial tissue biopsy absorbance (OD mg^{-1} tissue) of a) IgE b) IgG and c) IgA from cannulated animals while ○ infected with 4000 *T. circumcincta* d^{-1} (CIF) or ▲ similarly infected and immuno-suppressed (CISIF).

5.3.15. Serial tissue cell counts

Mean mast cell, globule leukocyte and eosinophil numbers observed in serial tissue biopsy samples are shown in Figure 5.14. There was an immuno-suppression x time interaction ($p=0.001$) for mast cell numbers, since cell counts in both groups increased throughout the trial, but at a greater rate in CIF animals.

An immuno-suppression x time interaction was also observed for globule leukocyte numbers ($p<0.001$), since globule leukocytes were detected only in CIF in increasing numbers from day 49.

There was an immuno-suppression x time interaction ($p<0.001$) in eosinophil numbers reflecting greater cell numbers in CIF than CISIF animals from day 14 to 70. Thereafter numbers in CIF were similar to those in CISIF until an increase in CIF but not CISIF on day 86.

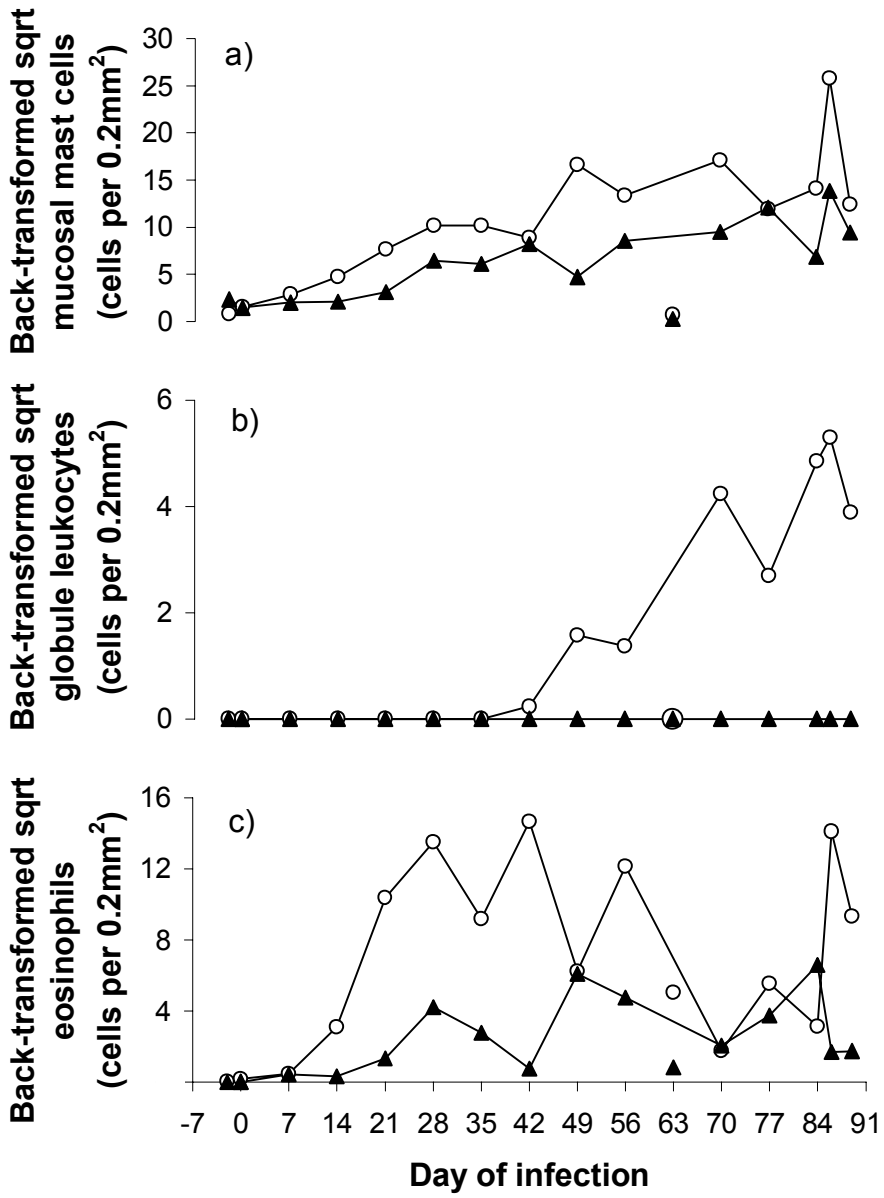

Figure 5.14: Mean back-transformed (sqrt (count + 1)) serial tissue biopsy cell counts per 0.2mm^2 of a) mucosal mast cells b) globule leukocytes and c) eosinophils from cannulated animals ○ infected with 4000 *T. circumcincta* d^{-1} (CIF) or ▲ similarly infected and immuno-suppressed (CISIF). The aberrant results from tissue samples collected on day 63 are shown with similar symbols but are not connected by lines.

5.3.16. Tissue antibodies at slaughter

Tissue antibody levels from the abomasum of cannulated animals at slaughter are displayed separately for the fundic and pyloric regions in Table 5.5. Overall, tissue IgE at slaughter was affected by sample site (p=0.012), with tissues from the pyloric region of CIF animals having an IgE absorbance 2.7 times that of those from the fundic region, while immuno-suppression had no significant effect in either fundic or pyloric tissue.

Overall, tissue IgG was affected by region (p=0.005), with absorbencies from pyloric tissue 1.6 times those of fundic tissue. Tissue IgG was not affected by immuno-suppression (p=0.854).

For IgA there was an effect of immuno-suppression (p=0.039)and region (p<0.001); the former was reflected in a 1.6 fold greater IgA absorbance in tissue collected from immuno-suppressed animals, while the latter reflects absorbance in pyloric tissue being 2.6 and 4.9 times greater than in those from fundic tissue in CISIF and CIF animals, respectively.

Table 5.5: Mean IgE, IgG and IgA absorbance (OD mg^{-1} tissue) of biopsy tissue collected from the fundic or pyloric regions of the abomasum at slaughter of cannulated animals infected with 4000 *T. circumcincta* d^{-1} (CIF) or similarly infected and immuno-suppressed (CISIF).

	Fundic		Pyloric		
	CIF	CISIF	CIF	CISIF	s.e.m
IgE	0.012^{a}	0.016^{ab}	0.033^{c}	0.026^{cb}	0.0055
IgG	0.018^{a}	0.020^{a}	0.030^{b}	0.030^{b}	0.0035
IgA	0.017^{a}	0.044^{a}	0.083^{b}	0.113^{b}	0.0128

^{a,b,c}Within each row, values with different superscripts are significantly different (p<0.05)

5.3.17. Abomasal tissue cell counts at slaughter

Mean mast cell, globule leukocyte and eosinophil concentrations in abomasal tissue at slaughter of non-cannulated and cannulated animals are shown in Table 5.6 and Table 5.7, respectively. Amongst non-cannulated animals, infection increased mast cell numbers four-fold in IF animals relative to C ($p<0.01$). Numbers of mast cells were similar for IS and C animals ($p=0.26$), and tended to be greater in ISIF animals relative to IS ($p=0.08$). However, immuno-suppression of infected animals did result in a 0.66 proportionate reduction in mast cells numbers ($p<0.01$). Immuno-suppression of cannulated animals resulted in a 0.42 relative reduction in mast cell numbers ($p=0.02$).

Both uninfected groups (C and IS) had no globule leukocytes present. Infection resulted in the appearance of globule leukocytes in IF animals ($p<0.01$), whereas concurrent immuno-suppression resulted in only a small rise in globule leukocytes in ISIF that was not different to IS animals ($p=0.47$). Immuno-suppression of cannulated animals prevented the rise in globule leukocyte numbers that was evident in CIF animals ($p=0.02$).

Eosinophils were not detected in C animals and only low numbers were found in IS animals. Infection resulted in an increase in eosinophils in IF animals ($p<0.01$) that tended to be proportionately suppressed by 0.27 in ISIF animals ($p=0.08$). Immuno-suppression reduced eosinophil numbers in CISIF relative to CIF animals by 0.39, although this was not significant ($p=0.18$).

Table 5.6: Sqrt-transformed (sqrt (count + 1)) mast cell, globule leukocyte and eosinophil cell counts per 0.2mm^2 from abomasal tissue at slaughter of non-cannulated animals infected with 4000 *T. circumcincta* d^{-1} (IF), similarly infected and immuno-suppressed (ISIF), immuno-suppressed only (IS) or maintained as controls (C). Back-transformed values are given in parenthesis.

	C	IF	IS	ISIF	s.e.m
Mast Cells per 0.2mm^2	0.67^a (0.5)	3.32^b (11)	0.23^a (0.1)	1.11^a (1)	0.44
Globule Leukocytes per 0.2mm^2	0^a (0)	0.8^b (1)	0^a (0)	0^a (0)	0.21
Eosinophils per 0.2mm^2	0^a (0)	2.62^b (7)	0.11^a (0)	1.92^b (4)	0.47

^{a,b}Within rows, values with different superscripts are significantly different ($p<0.05$)

Table 5.7: Sqrt-transformed (sqrt (count + 1)) mast cell, globule leukocyte and eosinophil cell counts per 0.2mm^2 from abomasal tissue at slaughter of cannulated animals infected with 4000 *T. circumcincta* d^{-1} (CIF) or similarly infected and immuno-suppressed (CISIF). Back-transformed values are given in parenthesis.

	CIF	CISIF	s.e.m
Mast Cells per 0.2mm^2	3.78^a (14)	2.20^b (5)	0.50
Globule Leukocytes per 0.2mm^2	1.3^a (1.6)	0^b (0)	0.39
Eosinophils per 0.2mm^2	2.53^a (6)	1.98^a (4)	0.40

^{a,b}Within rows, values with different superscripts are significantly different ($p<0.05$)

5.3.18. Small intestinal cell counts at slaughter

No globule leukocytes were found in any of the small intestinal tissue samples. Mean mast cell and eosinophil concentrations of small intestinal tissue retrieved at slaughter from non-cannulated and cannulated animals are shown in Tables 5.8 and Table 5.9, respectively. Amongst non-cannulated animals, mast cell numbers were similar in C and IF animals ($p=0.31$). Compared to their immunologically capable counterparts, mast cell numbers were similarly proportionately depressed by 0.88 in IS ($p<0.01$) and 0.83 in ISIF ($p<0.01$) animals and were not different. Similarly, immuno-suppression did not influence mast cell numbers in cannulated animals ($p=0.44$).

Amongst non-cannulated animals, eosinophil concentrations were proportionately increased by 0.40 in IF animals compared with controls (C) ($p<0.001$), whereas neither IS nor ISIF were significantly different to C animals ($p>0.05$). Immuno-suppression of cannulated animals did tend to comparatively reduce eosinophil numbers by 0.22, although this was not significant ($p=0.18$).

Table 5.8: Sqrt-transformed (sqrt (count + 1)) mast cell and eosinophil cell counts per 0.2mm^2 from small intestine tissue at slaughter of non-cannulated animals infected with 4000 *T. circumcincta* d^{-1} (IF), similarly infected and immuno-suppressed (ISIF), immuno-suppressed only (IS) or maintained as controls (C). Back-transformed values are given in parenthesis.

	C	IF	IS	ISIF	s.e.m
Mast Cells per 0.2mm^2	2.11[a] (4)	1.87[a] (3)	0.26[b] (0)	0.38[b] (0)	0.33
Eosinophils per 0.2mm^2	4.09[a] (17)	5.72[b] (33)	3.81[a] (15)	3.77[a] (14)	0.33

[a,b]Within rows, values with different superscripts are significantly different ($p<0.05$)

Table 5.9: Sqrt-transformed (sqrt (count + 1)) mast cell and eosinophil cell counts per 0.2mm^2 from small intestine tissue at slaughter of cannulated animals infected with 4000 *T. circumcincta* d^{-1} (CIF) or similarly infected and immuno-suppressed (CISIF). Back-transformed values are given in parenthesis.

	CIF	CISIF	s.e.m
Mast Cells per 0.2mm^2	3.39[a] (12)	3.27[a] (11)	0.53
Eosinophils per 0.2mm^2	6.37[a] (41)	5.66[a] (32)	0.51

[a]No significant differences within rows were observed ($p>0.05$)

5.4. Discussion

The role of the cannulated animals in this trial was to provide serial biopsy samples that would allow further interrogation of the changes occurring at the mucosal level during immunological development. It was anticipated that changes in productivity observed in non-cannulated animals may be reflected in immunological changes in the serial biopsy samples and allow an insight into the component of the immune response that incurs a productive cost to the animal. For this to be effective, the timing of events observed in the cannulated animals would need to be synchronised with those observed in non-cannulated animals indicating a similar response to infection. In general, this approach can be considered to have been effective as cannulated and non-cannulated animals had comparable FEC, total egg excretion and serum immunological profiles. However, at the time of slaughter on day 63, cannulated animals were maintaining a higher FEC of 360epg and were deemed to have not completed their immunological development and, as a consequence were slaughtered at the later date.

These results support the hypothesis that corticosteroid induced immuno-suppression alleviates the reduction in intake and production losses associated with infection of the abomasal parasite *Teladorsagia circumcincta*. They are therefore consistent with the observations during infection with the intestinal parasite *Trichostrongylus colubriformis* in Chapters 3 and 4, and provide support for the hypothesis the immune response *per se* is the major contributor to the cost of infection, and probably is a universal phenomenon of infection of different sites of the gastrointestinal tract and with different genera of nematodes.

The current infection regime can be considered to have been effective at providing a pathogenic infection. Despite the maintenance of low concentrations of nematode eggs in the faeces in infected (IF) animals from day 35 (Figures 5.4 and 5.5) indicating a rapid development of immunity compared

with the six to eight weeks for its development observed in previous studies utilizing similar infection regimes (Symons *et al*., 1981; Coop *et al.*, 1982), IF animals did appear to suffer pathological consequences of infection such as reduced serum albumin and a temporary reduction in feed intake that culminated in a 0.33 proportional depression in liveweight gain. Hypoalbuminaemia is indicative of enteric plasma protein leakage into the alimentary tract as a consequence of abomasal damage which is not replaced by commensurate synthesis (Symons *et al*., 1981). The proportional reductions in serum albumin of up to 0.10 in IF and 0.24 in CIF animals in the current study (Figure 5.7) are comparable with the peak reductions of 0.13 found by Symons *et al*. (1981) and 0.26 observed by Coop *et al*. (1982) during eight weeks of infection with 5000 *O. circumcincta* d^{-1}, suggesting 'normal' levels of abomasal pathology. In contrast, abomasal pH, which is commonly greater than pH 6.0 after day 20 of infection, as a consequence of decreased acid production coupled with an increase in secretory products due to damage caused to the gastric glands by the developing larvae (McLeay *et al*, 1973; Anderson *et al*., 1976; Titchen and Anderson, 1977; Anderson *et al*, 1981), was not increased in CIF animals until day 49 (Figure 5.12), although the abomasal pH of all infected groups was elevated at slaughter. Furthermore, although operating for only 14 days, the 0.17 proportionate reduction in feed intake of IF animals (Figure 5.2) was similar in magnitude to the 0.15 and 0.16 reduction observed for a period of six to eight weeks by Symons *et al*, (1981) and Coop *et al*. (1982), respectively. In spite of this short period of intake depression, the 0.33 relative reduction in fasted liveweight gain in IF animals (Figure 5.3) is comparable to the 36 and 0.40 depressions after 12 weeks of infection observed by Symons *et al*. (1981) and Sykes and Coop, (1977), respectively, and confirms that the larval dosing regime used in the current study was effective at providing a pathogenic infection.

Immuno-suppression reduced the nutritional disturbance of infection. Infection itself resulted in a 0.20 proportional reduction in gross efficiency of metabolizable energy (ME) utilization in IF animals (Table 5.4) that was comparable to the 0.28 observed by Sykes and Coop (1977) during infection with 4,000 *O. circumcincta* d^{-1} and the 0.19 found by Coop *et al*. (1982) during infection with 3,000 *O. circumcincta* d^{-1}. In contrast, ISIF animals experienced no reduction in gross efficiency of ME utilization, as has been observed in infections with *T. colubriformis* when immune function was suppressed (Chapter 3). The 0.46 proportional reduction in muscle deposition in IF animals may represent a shift of energy and protein synthesis from productive functions to support the production of acute phase proteins involved in the immune response and repair of the gastrointestinal tract (MacRae, 1993; Yu *et al*., 2000; Colditz, 2002). Interestingly, ISIF animals did not experience any reduction in muscle deposition other than what can be explained by immuno-suppression alone (Paranetto, 1979; Huang *et al*., 1998; Turini *et al*., 2003) and was observed in IS animals, suggesting the supply of protein for productive functions was not affected by infection in immuno-suppressed animals. Alternatively, the reduction in muscle deposition in IF animals may also reflect a reduced protein supply through a reduction of apparent N digestibility as a consequence of increased endogenous losses and/or decreased protein digestion (Sykes and Coop, 1977) that may not have been experienced by ISIF animals. Evidence for decreased endogenous N loss can perhaps be seen from the lack of a reduction in serum albumin in ISIF and in CISIF animals, indicating reduced plasma protein leakage as a result of abomasal pathology, and is supported by the studies of Lawerence *et al*. (2001) who observed mast cell deficient mice appeared to suffer reduced mucosal pathology in the form of reduced villi length despite harbouring a much larger worm burden after 13 days of infection with *T. spiralis*. On the other hand, recent studies utilizing Cr^{51} appearance in the faeces of sheep receiving a mixed infection of *T. circumcincta* and *T. colubriformis* have suggested that the net loss of plasma protein is not significantly reduced in immuno-suppressed animals (Vaughan, 2005).

Therefore, the ability of ISIF and of CISIF animals to maintain serum albumin concentrations seems likely to have been due to an enhanced ability to replace plasma proteins that had leaked into the alimentary tract. Evidence for this can be observed in the larger livers of immuno-suppressed animals that may suggest a greater ability for the production of constitutive and export proteins (Husband and Bryden, 1996) from amino acids that were made available through the net catabolic effects of corticosteroids (Turini *et al.*, 2003). This notion of a net surplus of amino acids is supported by the greater serum urea concentrations of ISIF and CISIF, indicative of deamination of amino acids. Therefore, it is likely that the apparent minimal disruption to protein deposition in ISIF animals was a consequence of reduced partitioning of protein to immunological functions or the repair of damage to the gastro-intestinal tract that IF animals could not avoid. These results add support to the theory that the metabolic disturbance caused during infection with the abomasal nematode *T. circumcincta* is primarily a reflection of the nutritional cost of the immune response, and not a consequence of abomasal pathology caused by the parasite *per se*.

Feed intake in IS animals in the current study did tend to be increased compared with C animals (Figure 5.2). Previous use of methylprednisolone acetate at the same dose rate used here in Chapters 3 and 4 had no effect on feed intake, however, corticosteroid administration to sheep appears to have a varied effect on feed intake that is dependant on the dose given. There is no evidence in the literature of the effect of methylprednisolone acetate on appetite in sheep. However, single doses of 0.1mg kg^{-1} of the corticosteroid dexamethasone (equivalent to 0.5mg kg^{-1} methylprednisolone) have been shown to stimulate short term increases in food intake (Adams and Sanders, 1992), while larger single doses of 6mg kg^{-1} dexamethasone have been shown to cause a short term decrease in feed intake (Paranetto, 1979). The apparently greater feed intake of IS compared with C animals may, alternatively, be a consequence of an apparent reduction in appetite experienced by C animals

from day 35 for reasons that are not evident, but can be mostly attributed to a 0.50 proportional reduction experienced by one C animal from this point. Nevertheless, infection in addition to immuno-suppression did not result in any depression of intake, with ISIF consuming food at rates similar to IS animals at all times despite harbouring a considerable parasite burden. While specific evidence is not available for ruminants, human and murine studies have identified pro-inflammatory cytokines produced by the developing immune response, including interleukin (IL)-1, IL-6, IL-8 and tumour necrosis factor (TNF)-α, that act on the appetite centres in the hypothalamus through the afferent pathway causing an anorectic effect (Johnson, 1998; Langhans, 2000; Farthing and Ballinger, 2001). Although cytokine production was not measured in the current study, it is highly probable the corticosteroid treatment in ISIF and in CISIF animals interrupted the development of the immune response at a level which modified the production of appetite depressing pro-inflammatory cytokines that may have been responsible for the temporary 0.17 relative depression of appetite in IF and prolonged lower levels of feed intake in CIF animals. Furthermore, these results provide evidence that the alleviation of the parasite-induced anorexia in young lambs with corticosteroids could be a universal phenomenon of nematode parasitism of the gastrointestinal tract.

Treatment with corticosteroids appeared to have variable effects on parameters of immune function that may question the effectiveness of this treatment regime to provide effective immuno-suppression. While the elevated eosinophils in the small intestine of IF animals (Table 5.8) may indicate some level of immunological priming, possibly due to a downstream effect of either antigenic stimulation or the release of immunological secretions (Stewart, 1955), the lower numbers of mast cells and eosinophils in both IS and ISIF animals suggest that corticosteroid treatment had a systemic immuno-suppressive effect. However, this was not reflected in the small intestine cell counts of cannulated animals (Table 5.9). Furthermore, despite both ISIF and CISIF animals exhibiting a considerably greater faecal nematode egg output, neither

serum antibodies (Figures 5.10 and 5.11) nor tissue IgE or IgG (Figure 5.13) were reduced by corticosteroid treatment. There are no reports in the literature regarding the effect of corticosteroids on antibody responses of sheep during abomasal infections; however, weekly treatment with the corticosteroid dexamethasone has previously been shown to be effective at suppressing serum antibody production specific to the intestinal parasite *T. colubriformis* (Douch *et al*., 1994). In the current series of experiments, methylprednisolone acetate has not always resulted in a reduction in *Trichostrongylus colubriformis* specific IgA, but has consistently been effective in suppressing total antibody production (Chapters 3 and 4). For reasons that are unclear, IgA was elevated in both fundic and pyloric tissue at slaughter of CISIF animals (Table 5.5), but did appear to be successfully suppressed in serial biopsy tissues (Figure 5.13). Furthermore, changes in tissue IgA (Figure 5.13) from both CIF and CISIF animals did not appear to be reflected in serum levels (Figure 5.11). The discrepancy between serum IgA and serial tissue IgA has been observed previously in sheep by Sinski *et al*. (1995) who found that only 0.25 of the variation in plasma IgA could be accounted for by the IgA in mucosal scrapings after immunologically experienced lambs were given a challenge dose with *O. circumcincta*. Rinkinen *et al*. (2003) concluded serum IgA was a poor indicator of mucosal secretory IgA from duodenal brush samples from healthy dogs of various breeds. In addition, Nara *et al*. (1983) found serial jejunum biopsy samples from dogs infected with canine parvovirus had no correlation with serum IgA, IgM and IgG. Interestingly, immuno-suppression in the current study drastically reduced the production of tissue L3 IgA while apparently not causing any diminution in serum L3 IgA; suggesting serum IgA in immuno-suppressed animals does not have a mucosal origin. Schneider *et al*. (1998) came to the same conclusion in immuno-compromised HIV infected humans since elevated serum IgA in patients that were HIV positive was not reflected through increased mucosal IgA. Differentiation of bovine mucosal B cells into plasma cells and the subsequent immunoglobulin production, including IgA, has been shown to be regulated through the cytokines IL-1 and IL-2 (Collins

and Oldham, 1995) which would be expected to be suppressed as a consequence of corticosteroid treatment. This may not have been reflected in a decrease in serum IgA in immuno-suppressed animals as although it may be expected that levels observed peripherally in the serum would be indicative of those at the site of production, cells engaged with in the production of mucosal IgA do not contribute significantly to the circulatory pool (Mestecky *et al*., 1999). Further evidence for successful effects of immuno-suppressive treatment in the current study can be observed from the reduction in both serial (Figure 5.14) and terminal (Tables 5.6 and 5.7) mucosal mast cell and eosinophil counts and apparent abolishment of globule leukocyte production in abomasal tissue. Moreover, the six-fold greater worm burden at slaughter of corticosteroid treated animals provides strong evidence to suggest that the regime of corticosteroid treatment was successful at suppressing the effecter arm of the immunological reaction. This aside, the discrepancy between serum and tissue IgA is of concern, as much of our understanding of immunological function during gastro-intestinal parasite infections has been derived from measuring systemic immunoglobulins in serum, which do not appear to be reflective of the changes occurring at the site of parasitic infestation.

Immuno-suppression of infected animals allows the separation of parasite population dynamics from those which are immunologically mediated via the host. Adult nematode populations are undoubtedly influenced by the immune status of the host animal, reflected in lower worm burdens in IF and CIF animals at slaughter and which can be attributed to the development of an effective immune response (Tables 5.2 and 5.3). However, adult *T. circumcincta* populations are also considered to be regulated by the incoming L3 larvae (Barger, 1987). This later notion is supported by the studies of Donaldson *et al*. (2001) who found the worm burdens in *post partum* ewes at slaughter 10 days after a challenge infection of 25,000 *T. circumcincta* and 17,500 *T. colubriformis* in addition to a trickle infection of 10,000 *T. circumcincta* and 7,000 *T. colubriformis* d^{-1} had fewer adult worms but greater numbers of L4 larvae compared to the

burdens retrieved from ewes that received the trickle infection only. Evidence for such an effect in the current study can be observed from the comparable worm burdens at slaughter of both ISIF and CISIF animals suggesting adult parasite populations in these animals had reached a ceiling that was reflected in a plateau in total egg production (Figure 5.5), despite the latter receiving 100,000 more larvae due to an extended infection period. This concept is supported by Symons *et al*, (1981), who found increasing larval dose rate from 37,500 to 120,000 *O. circumcincta* week^{-1} resulted in an increase in worm burden at necropsy after eight weeks of infection from 22,300 to only 31,200. Population dependant regulation of egg production in *T. circumcincta* is a phenomenon that is well established (Symons *et al*., 1981; Callinan and Arundel, 1982; Barger, 1987; Donaldson *et al*., 2001). Calculation of mean daily egg excretion indicates egg production was 0, 13, 25 and 37 eggs per L5 female worm d^{-1} for IF, ISIF, CIF and CISIF groups, respectively, at the time of slaughter. Greater fecundity of worms from CISIF compared with ISIF may be explained through adult L5 female density dependant regulation, however, the larger worm burdens and greater fecundity of worms from immuno-suppressed hosts provides evidence that host immunological mechanisms also have a strong influence on the suppression of egg production. Worm fecundity in *O. circumcincta* infection has been closely associated with worm length (Stear *et al*., 1999) which has been observed to have a strong negative correlation (R^2=0.92) with peak lymph IgA (Smith *et al*., 1983). In addition, worm length of *O. circumcincta* has also been indicated to be regulated through density dependant mechanisms (Callinan and Arundel, 1982; Donaldson *et al*., 1998). However, it is not known if this can be considered a true density dependant regulatory effect, or is the consequence of an enhanced IgA response through greater antigenic stimulation from a greater worm burden. The effects of the greater worm burden on worm length in immuno-suppressed animals in the current study were variable, with a non-significant decrease in ISIF and a marginally non-significant increase in CISIF animals. Consequently, no clear conclusion can be drawn on the extent of density dependant regulation of

worm length or fecundity. Alternatively, these results suggest that worm length was regulated by components of the immune response that were not suppressed by corticosteroid treatment. Evidence for this can be seen in the comparable serum L3 IgA profiles between IF and ISIF and between CIF and CISIF (Figure 5.11), although female worm length and peak serum IgA were not well correlated (R^2=0.005). Furthermore, these results do not support the suggestion that worm length is closely associated with worm fecundity. Interestingly, the greater worm burdens in immuno-suppressed animals were accompanied by an increase in the proportion of inhibited L4's. Inhibition of L4 larvae is considered to reflect the development of host resistance to incoming larvae (Douch, 1989; Seaton *et al*., 1989), although it has been observed not to be affected by protein supplementation which was effective at reducing worm burdens in the periparturient ewe (Donaldson *et al*., 2001). Removal of adult populations in calves through anthelmintic treatment has been shown to stimulate the development of arrested L4 *Ostertagia ostertagi* larvae (Michel, 1971), suggesting adult population density-dependant mechanisms may also be involved. In addition, Michel (1970) found higher larval dose rates of *Ostertagia ostertagi* in calves resulted in greater worm burdens in addition to an increased proportion of larval inhibition at the fourth stage. However, it is unclear if inhibition was influenced through density-dependant mechanisms from adult parasites or if the larger dose rate caused a greater antigenic stimulation of the immunological response. Immuno-suppressed animals in the current study exhibited no evidence of an enhanced immunological response; therefore the greater proportion of inhibited L4 larvae may be attributed to parasite density dependant regulation.

The use of abomasal cannulae in the current trial allowed repeated retrieval of abomasal biopsy samples to examine cellular changes during immunological development. Serial biopsy samples taken in this manner have been shown to be generally indicative of cellular change occurring in larger tissue samples (Pfeffer *et al*., 1996; Huntley *et al*., 2004). In particular, mast cells have been

reported to be uniform in distribution in the abomasum while that of globule leukocytes and eosinophils has been observed to be more variable, with the latter tending to suggest focal proliferation, rather than a unified whole organ response (Huntley *et al*., 1992a). This may provide an explanation for the similar numbers of mast cells, but not globule leukocytes or eosinophils in tissue taken at slaughter (Table 5.7) compared to previous serial biopsy samples (Figure 5.14) from CIF and CISIF animals.

The development of resistance to nematode establishment and the elimination of adult worm burdens are associated with marked cellular infiltration of the intestinal mucosa with mast cells and globule leukocytes (Miller and Jarret, 1971) that are believed to have the same cell lineage (Huntley, 1992). The slight reduction in mucosal mast cells but apparent abolishment of globule leukocytes in serial biopsy tissue samples in CISIF compared with CIF suggests that the immuno-suppressive treatment prevented the maturation of mucosal mast cells to globule leukocytes, thus preventing parasite expulsion and reduced egg output typically associated with an effective immunity. A similar effect of corticosteroid treatment on cell proliferation was observed by Huntley *et al*. (1992b) as dexamethasone treatment reduced mast cell numbers by proportionately 0.50 while preventing the development of any globule leukocytes in naïve sheep after a challenge dose with *H. contortus*. In slight contrast, Douch *et al*. (1986) observed that dexamethasone treatment abolished both mucosal mast cells and globule leukocytes in the small intestine of sheep that were trickle infected with *T. colubriformis*. Typically, increases in both mucosal mast cells and globule leukocytes have been associated with immunological protection (Douch *et al*., 1983; Douch *et al*., 1988; Stankiewicz *et al*., 1993; Huntley *et al*., 1995), the functional activity of which is considered to be mediated through IgE (Lewis and Austen, 1981; Huntley, *et al*., 1995). Interestingly and surprisingly, IgE concentrations in serial tissue biopsies did not appear to be affected by immuno-suppression (Figure 5.13). Nevertheless, it is apparent from the cell counts of both terminal tissue (Tables 5.6 and 5.7)

and the rise in globule leukocytes in serial tissue (Figure 5.14) as total egg production declined, that a protective immunity in the current trial was more closely associated with the appearance of globule leukocytes than mucosal mast cells.

The mass proliferation of proteinacous cells during the development of immunity would be expected to carry a considerable nutritional penalty and reduced animal performance through competition between productive functions and the immune response for rate-limiting nutrients (MacRae, 1993; Coop and Holmes, 1996; Coop and Kyriazakis, 1999). Indeed, globule leukocytes act through larval migration inhibitory activity in the mucus that is mediated through the slow-reacting substance of anaphylaxis (SRS-A) (Douch *et al*., 1986), the major constituent of which are cysteine rich leukotrienes (Lewis and Austen, 1981), and if they are produced in a greater quantity during the development or maintenance of immunity could reduce the net availability of this sulphur-amino acid for other tissues (MacRae, 1993). Support for this can be observed from the studies of Douch *et al*., (1986) and Stankiewicz *et al*. (1993), both of whom found globule leukocytes to be more prominent in the abomasal and intestinal mucosa of animals selected to be genetically resistant to gastro-intestinal parasites that have repeatedly been shown to have reduced productivity during nematode challenge compared to their unselected counterparts (Watson *et al*., 1992; Morris *et al*., 1997; Morris *et al*., 2000). Therefore, the increase in mast cell and globule leukocyte numbers in CIF animals from day 42 would also be expected to cause production losses through competition for potentially rate-limiting amino acids. However, the comparable liveweight gain of CIF to CISIF animals from day 42 until the conclusion of the trial (Figure 5.3) indicates the production of mast cells and globule leukocytes were not associated with reductions in animal performance. Indeed, the productive cost of immunological development in CIF animals reflected in liveweight stasis appeared to occur in the three-week period prior to the appearance of a globule leukocyte response. These results support the

observation in Chapter 4 that the disturbance to productivity in young lambs does not appear to be associated with mechanisms of the immune response that are directly involved in the expulsion of parasites.

In summary, these results demonstrate that corticosteroid-induced immuno-suppression prevents the reduction in nutrient utilization and reduction in feed intake in young lambs during infection with the abomasal nematode *T. circumcincta*. Furthermore, these findings support the hypothesis of the developing immune response being the major contributor of production losses in young parasitised lambs and provide evidence that this effect is a universal phenomenon for both abomasal and intestinal infections.

Chapter 6

General summary

The results of this series of experiments provide clear evidence that the host immune response to gastrointestinal nematode infestation in sheep incurs a considerable nutritional cost. Proportionate reductions in the gross efficiency of metabolizable energy (ME) utilization as a consequence of infection in immunologically normal animals varied from 0.16 in ewes with an established immune response (Chapter 3) to 0.50 in young lambs offered a low protein diet (Chapter 4). In contrast, regardless of the site of parasite infestation (small intestine or abomasum), lambs that were concurrently immuno-suppressed and infected did not suffer any reduction in nutrient utilization other than that which could be attributed to the effects of immuno-suppression alone. The reduction in nutrient utilization in infected animals has been attributed to an increase in endogenous losses through leakage of plasma proteins and sloughing of the epithelial cells as a consequence of abomasal and intestinal pathology (Coop and Holmes, 1996; van Houtert and Sykes, 1996) coupled with a reduction in feed intake that has the effect of increasing the proportion of nutrients used for maintenance, thus reducing the gross efficiency of utilization of ME for production. There was, however, evidence for direct pathological damage by the nematode which was not removed by immuno-suppression, as shown by comparable reductions in serum phosphate of ISIF animals to that of IF animals (Chapters 3 and 4) and similar abomasal pH at slaughter of ISIF to that of IF animals during abomasal infections (Chapter 5). On the other hand, immuno-suppressed animals did not appear to suffer a reduction in serum albumin that was consistently exhibited by immunologically normal animals. Recent studies investigating this aspect by measurement of the appearance of intravenously administered Cr^{51} in the faeces have indicated that immuno-suppression does not prevent the increase in plasma protein leakage (Vaughan, 2005). Together these allow the conclusion that the absence of a reduction in

serum albumin in immuno-suppressed animals was a consequence of an enhanced ability to replace lost protein and not as a consequence of reduced pathological damage. However, the consistently greater intakes of ISIF animals compared with IF prevent the calculation of the true nutritional cost of immune development on nutrient utilization. Estimates of the true cost of immunity would require the use of pair-fed animals, in which case the proportion of energy utilized for maintenance would not be affected by the parasite-induced reduction in appetite. Nevertheless, the lack of a reduction in ME utilization in animals concurrently immuno-suppressed and infected provides clear evidence that both *T. circumcincta* and *T. colubriformis* nematodes *per se* cause very little metabolic disturbance, and that a majority of the reduction in nutrient utilization may be attributed to the physiological changes associated with the developing immune response and its associated nutritional demands.

The ubiquitous reduction in feed intake as a consequence of gastrointestinal nematode parasitism in young sheep can be considered to be the single most important factor contributing to reduced animal performance (Sykes, 1994), contributing up to 0.90 of the observed production losses (Dargie, 1980; Sykes, 1987; van Houtert and Sykes, 1996). Consequently, the cause and reason of this aspect of parasitism has received considerable research attention (Symons and Hennessy, 1981; Dynes *et al*., 1990; Kyriazakis *et al*., 1994; Kyriazakis *et al*., 1996; Dynes *et al*., 1998; Kyriazakis *et al*., 1998), although the mechanisms involved are still not known. One of the most salient findings of these studies was that despite harbouring large parasite burdens, animals which were concurrently immuno-suppressed did not suffer a reduction in feed intake. Furthermore, the consistent reduction in appetite in nematode naïve lambs and the absence of a reduction in immunologically competent ewes (Chapter 3) suggests that component(s) of the developing immune response may be involved in the occurrence of anorexia in sheep infected with gastrointestinal nematodes. Moreover, studies with the intestinal parasite *T. colubriformis* in Chapters 3 and 4 indicated that the reduction in intake may be associated with the production

of immunoglobulin (Ig) A. In Chapter 3, a rise in serum IgA was observed in IF lambs and in the aberrant individual (No55), both of which also suffered a reduction in appetite. In contrast, ISIF lambs, IF ewes and ISIF ewes displayed neither an increase in serum IgA nor a reduction in appetite. Further support for the association of IgA with the reduction of appetite can be obtained from Chapter 4, as those animals that were classed as IgA responders suffered a greater reduction in appetite than those classed as IgA non-responders. However, the association between serum IgA and a reduction in feed intake was not apparent during infection with the abomasal parasite *T. circumcincta* (Chapter 5), while the same pattern of reduced feed intake in IF but not ISIF animals was observed, both groups displayed similar serum IgA profiles. This could possibly reflect a lower level of immunological priming during abomasal infections compared with those of the intestine (Douch *et al.*, 1996b). On the other hand, IgA concentrations in serial biopsy tissue samples from cannulated animals did indicate that production of IgA local to the site of parasite infestation in the abomasal mucosa was suppressed in corticosteroid treated animals. The reasons for the discrepancy between tissue IgA and that found in the serum are unclear as it may have been expected that the nematode-specific immunoglobulins found in the serum would have originated from the site of the mucosa-parasite interface. Nevertheless, these results indicate that the association between IgA and anorexia during abomasal infections cannot be discarded. While neither a direct, nor functional basis for the association between IgA and reduced intake can be provided, it may be possible to speculate that pro-inflammatory cytokines of the developing immune reaction are involved, in particular IL-6 which is known to be associated with IgA production (Ramsay *et al.*, 1994; Husband *et al.*, 1996). Cytokine measurement was not available for these studies, but it is probable that immuno-suppression influenced either the production of pro-inflammatory cytokines, or their effect on the appetite centres in the hypothalamus (Spurlock, 1997; Johnson, 1998). In support of this, Dynes *et al.* (1990) observed a short term increase in intake when brotizolam was used to block satiety signals at the ventromedial

hypothalamus. It is evident that further investigation into the role of pro-inflammatory cytokines in the reduction of appetite experienced by young lambs during the development of immunity is required to elucidate the mechanisms involved.

While it may be apparent from this series of experiments that component(s) of the developing immune response are involved in the reduction of appetite, this does not explain why animals have evolved a mechanism which reduces nutrient intake at a time when demands for energy and protein for the development of immune function and repair of damaged tissue are increased. In an attempt to find a functional basis for the evolution of this apparently illogical and nutritionally disruptive phenomenon, Kyriazakis *et al*. (1998) suggested that anorexia during parasitic infection may be viewed as a disease coping strategy that aids the recognition of the parasite by the immune system, and that strategies to complement it should be considered to allow greater resilience to the effects of infection. While it is still undetermined if anorexia does promote a beneficial immune response, the absence of both anorexia and loss of performance in immuno-suppressed and infected animals suggests a depression in feeding behaviour during nematode infection is not essential for the animal to express greater resilience to cope with infection. Furthermore, the greater reduction in feed intake experienced by LIF compared with HIF animals (Chapter 4) did not appear to provide any advantage for the development of immunity. Alternatively, the additional protein received by HIF animals may have altered the immunological cytokine cascade to one that was less metabolically disruptive. Although there still may be some question regarding the exact ovine cytokine cascade, there is support for a Th-1/Th-2 dichotomy in sheep (Gill *et al*., 2000). It is hypothesised that the cytokine production during a Th-2 type reaction involved in an effective immune response down-regulates the production of the pro-inflammatory Th-1 cytokines, thus lessening the reduction in voluntary feed intake. This may provide a functional explanation for the reduced anorexia in high protein fed animals and the absence of reduced

intake in immuno-competent ewes, as these animals would be expected to have sufficient nutrient resources available to promote a Th-2 type immune reaction. Therefore, rather than being viewed as a disease coping strategy, the ubiquitous reduction in intake during gastrointestinal nematode infections in sheep may be an evolutionary hangover from the strong genetic selection of successive generations of sheep for productive traits that have not been matched by advances in animal nutrition through plant breeding, resulting in the diversion of nutrients away from immune function that may increase the susceptibility of animals to promote a pro-inflammatory Th-1 type immune reaction.

The ultimate aim of this research is to assist the development of sustainable control strategies for gastrointestinal nematode parasites. One method of achieving this that has received considerable attention is through aiding the development and maintenance of a natural immunity to parasites either through nutritional supplementation (Bown *et al*., 1991a; Coop *et al*., 1995; van Houtert *et al*., 1995; Donaldson *et al*., 1998; Houdijk *et al*., 2000; Donaldson *et al*., 2001; Houdijk *et al*., 2003; Houdijk *et al*., 2005), vaccination (Wagland *et al*., 1984; Douch *et al*., 1988; Stankiewicz *et al*., 1996; Hein *et al*., 2001; Knox *et al*., 2001; Meeusen and Piedrafita, 2003) or the selection of animals that are genetically predisposed to mount a strong immune reaction (Watson, *et al*., 1992; Bishop and Stear, 1997; Morris *et al*., 1997; Morris *et al*., 2000; Bisset *et al*., 2001; Morris *et al*., 2005). While this thesis has already provided evidence to suggest that strategic protein supplementation may reduce the disruption caused by immune signalling, the alternative methods of stimulating a strong immune reaction such as vaccination or selection of resistant animals may not result in more productive animals unless they are successful in promoting the development of a nutritionally economical immune response. This hypothesis is supported by the consistently poor production characteristics of animals that have been selected solely on their ability to limit FEC (Watson, *et al*., 1992; Morris *et al*., 1997; Morris *et al*., 2000, Morris *et al*., 2005), and the recent shift in breeding philosophy to the selection for animals that can promote an effective

immune response while maintaining high productivity (Bisset *et al.*, 2001; Morris *et al.*, 2004). The implications of this current series of experiments are that, once the detrimental component(s) of the immune response are identified, it may be possible to develop markers to assist either the development of short-term nutritional supplementation strategies, vaccination regimes or the selection of animals that are able to promote an effective immune reaction with a minimal metabolic cost.

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